JOURNAL OF DAIRY SCIENCE

MARCH, 1957

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ANNUAL MEETING

June 26-29, '57

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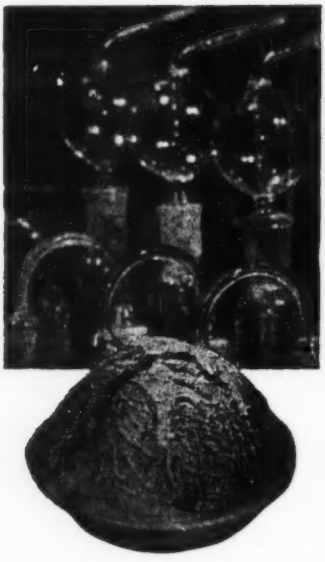
FRODEX

CORN SYRUP SOLIDS



A distinct and unique contribution to the technology of ice cream manufacturing since it was pioneered by American Maize some two decades ago, FRODEX (corn syrup solids) is still the corn sweetener most widely used by leading ice cream manufacturers.

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The many uses and growing advantages of FRODEX are the result of continuing research into fine products made from corn by American Maize-Products Company.

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from research laboratories

. . . experiment stations

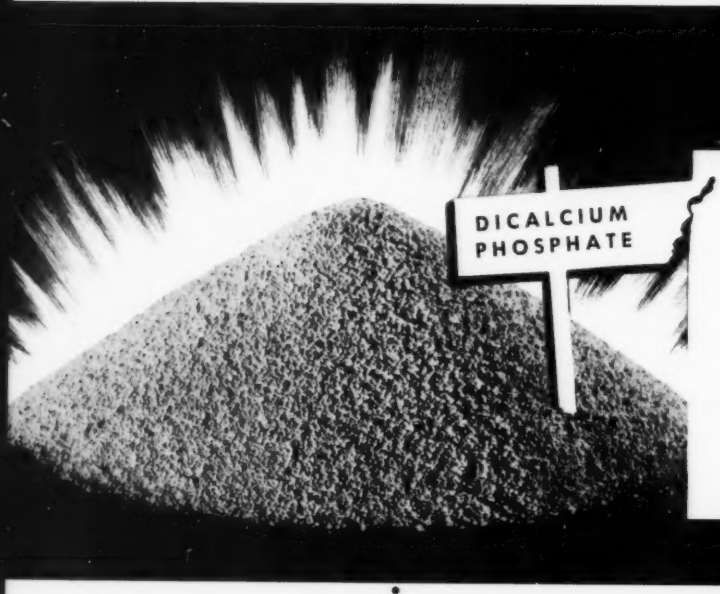
. . . feedlots

Research shows the penalties of feeding

"phantom" phosphorus

Recent data, reported on the following pages, reveal how "phantom" phosphorus penalizes feed performance . . . show there is a big difference in feed phosphorus sources.

Research shows why d
is today's superior p



Chemically processed and purified dicalcium phosphates are produced by reacting phosphoric acid with limestone. Such phosphates are uniform . . . consistently high in biological availability . . . low in fluorine. Newer research shows that dicalcium phosphate provides superior phosphorus nutrition for farm animals at lower cost.

Curacao Island Phosphate is an imported raw rock phosphate. Quality varies . . . averages less than 70% available phosphorus for chicks. High fluorine content limits its use in animal feeds. High "phantom" phosphorus content makes it an expensive source of feed phosphorus.

These by-products of the meat packing industry vary in biological availability. They may penalize feed performance with "phantom" phosphorus — phosphorus that farm animals can't utilize. Fluorine content is often high. Bone meal products also may add unnecessary cost to farm feeds.

Soft phosphates are raw, unprocessed rock products . . . often high in clay and silt. Fluorine content also is high . . . safe only in small amounts for short periods. Soft phosphates are low in biological availability . . . high in "phantom" phosphorus.

dicalcium phosphate phosphorus source

Works harder nutritionally than bone meal, Curacao, soft phosphates, or any other source containing "phantom" phosphorus*

Purdue University nutritionists recently boosted pig gains as much as ½ lb. per head daily . . . just by replacing a commonly used, raw phosphorus source with dicalcium phosphate.

Other university tests show similar results for cattle, chicks and poults.

The reason, say nutritional fact finders, is "phantom" phosphorus . . . phosphorus in the feed that's biologically out of reach for farm animals. It's the portion (often high) that is biologically unavailable.

Such evidence has led to a re-evaluation of feed phosphorus sources, including such old stand-bys as bone meal. It explains why so many feed manufacturers are now fortifying feeds with new, high-potency dicalcium phosphate products like International's superior quality Dynafos.

Dicalcium phosphates are chemically produced so nutritional quality can be carefully controlled. Fluorine content also can be held far below safe standards set up to protect animal health. And such phosphates eliminate any possible hazard of carrying disease.

Since phosphorus affects the performance of *all* other ingredients in a ration, the development of superior sources like Dynafos provides new profit opportunities for the farmer. It frees him from the penalties of "phantom" phosphorus . . . assures the faster gains and higher production that complete phosphorus nutrition can provide.

*"Phantom" phosphorus is the phosphorus from low-grade unprocessed phosphates that is biologically unavailable . . . out of reach of farm animals. It can't contribute to feed performance.

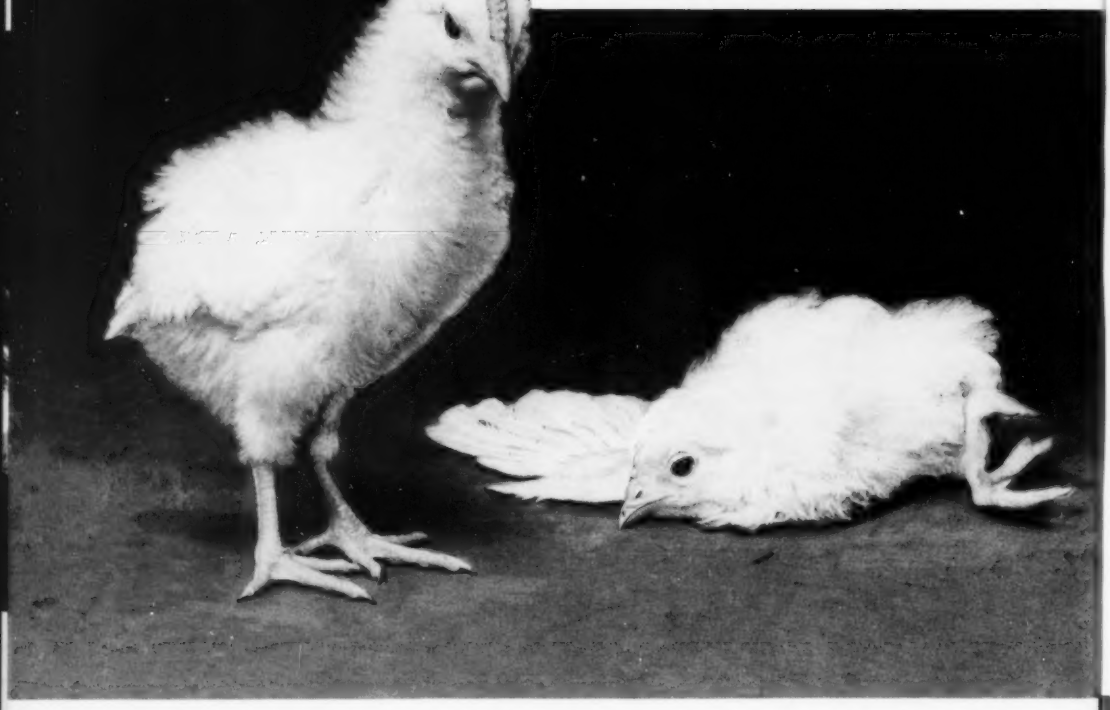
One pound of dicalcium phosphate supplies as much usable phosphorus as 2 to 9 pounds of unprocessed, raw phosphates . . .

SPECIFICATIONS	DYNAFOS	SOFT OR COLLOIDAL PHOSPHATE	CURACAO ISLAND PHOSPHATE	BONE MEAL
1 PHOSPHORUS CONTENT	18.5%	9.0%	14.0%	13%
2 DYNAMIC (BIOLOGICAL) VALUE	100	Less than 25	70	70-100
3 LBS. OF USABLE PHOSPHORUS PER TON	370 lbs.	Less than 45.0 lbs.	196 lbs.	182-260 lbs.
4 MAXIMUM FLUORINE CONTENT	1,200 ppm	15,000 ppm	5,000 ppm	600-8,760 ppm

International Minerals & Chemical

manufacturers of dynamic *DYNAFOS* dicalcium phosphate

"phantom" growth, boosts



Only the kind of phosphorus was different

"Phantom" phosphorus in poultry feeds may cause mild or severe phosphorus deficiencies. Such phosphorus acts as a nutritional barrier in feeds because it's biologically out of reach.

dynamic DYNA·FOS

International's dynamic Dynafos is a chemically produced dicalcium phosphate. Its superior biological availability and low fluorine content provide low-cost insurance against mild or severe phosphorus deficiencies.

phosphorus slows chick mortality, steps up costs

Dicalcium phosphate assures the higher biological availability and complete phosphorus nutrition modern poultry flocks require

Three-pound broilers in 6 weeks . . . 2.00 feed conversion! That's the goal today's broiler growers are shooting at. Other poultrymen expect better chick and poult growth . . . higher rate of lay.

Low-grade, unprocessed phosphates can't meet the stepped-up need for full, nutritional power. They shackle feed performance with "phantom" phosphorus that poultry and livestock can't use.

That's why dicalcium phosphate products . . . with their consistently higher biological availability . . . are used by more and more feed manufacturers today. Their greater nutritional power is shown by college tests.

For example, in recent studies at Ontario Agricultural College, dicalcium phosphate grew chicks nearly 25% faster than ground rock phosphate . . . on ½ lb. less feed per lb. of gain.

South Dakota State College studies show that dicalcium phosphate is 3 times more effective for poults than Curacao . . . at least 4 times more effective than soft phosphates.

Such research emphasizes how "phantom" phosphorus can penalize feed performance. It cancels the effectiveness of more expensive ingredients . . . cuts down profits.

Chemically processed and purified dicalcium phosphates — such as International's Dynamic Dynafos — meet this need for a harder-working feed phosphorus. It's a safe . . . sure . . . economical way to assure complete phosphorus nutrition.

Today's poultry need more phosphorus

To reach today's goal of 3 lbs. in 6 weeks, a broiler must convert nearly 79% more phosphorus every day to its bodily needs than a well-fed broiler of 15 years ago.

Layers need more phosphorus, too. An average hen makes 70 more trips to the nest than the average hen of 1935 . . . must convert 57% more phosphorus every day to the job of producing eggs and staying healthy.

International leads the way in phosphate research

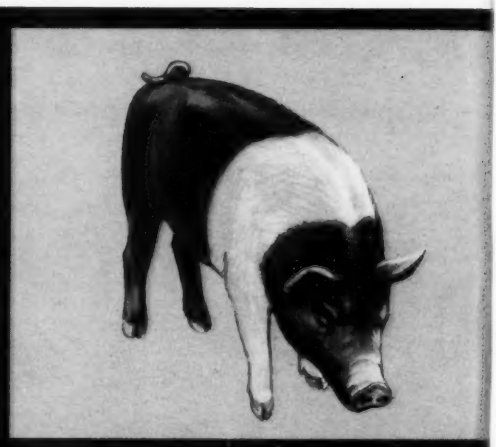
Each year International's scientists conduct more phosphorus research than the rest of the phosphate producers combined. The development of a superior dicalcium phosphate — dynamic Dynafos — makes the benefits of this research available to poultrymen and livestock producers.

MINERALS International CHEMICALS

International's research is geared to find the answers to all phases of phosphorus nutrition and production

"phantom" phosphorus n
down gains, cuts f

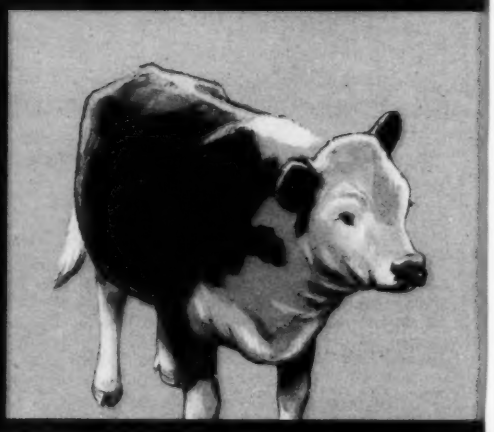
DICALCIUM PHOSPHATE
1.38 lbs. av. daily gain



SOFT PHOSPHATE
.84 lb. av. daily gain

At Purdue, pigs gained more than ½ lb. faster per day . . . on nearly ½ lb. less feed per lb. of gain . . . when dicalcium phosphate replaced soft phosphate.

.25% PHOSPHORUS
2.93 lbs. av. daily gain



.18% PHOSPHORUS
2.46 lbs. av. daily gain

At Iowa State College, fortifying fattening rations with additional dicalcium phosphate boosted gains nearly ½ lb. daily . . . cut 1.4 cents off the cost of each pound of gain.

s makes feeds lazy, slows s feed efficiency

New college work confirms that livestock need a harder-working phosphorus . . . dicalcium phosphate supplies it

Feeding trials at leading colleges and experiment stations show that feed phosphates containing "phantom" phosphorus can disable feeds nutritionally. And these same tests indicate that dicalcium phosphates deliver superior nutritional results.

When Iowa State College researchers fortified fattening rations with higher levels of dicalcium phosphate, they boosted cattle gains by an average of nearly ½ lb. daily.

In these tests steers fed a ration containing .18% phosphorus — the customary recommended level — gained 2.46 lbs. per day at a cost of 16.4 cents per pound. But when dicalcium phosphate raised the phosphorus level to .25%, the steers gained 2.93 lbs. per day. The cost per pound of gain dropped to 15 cents.

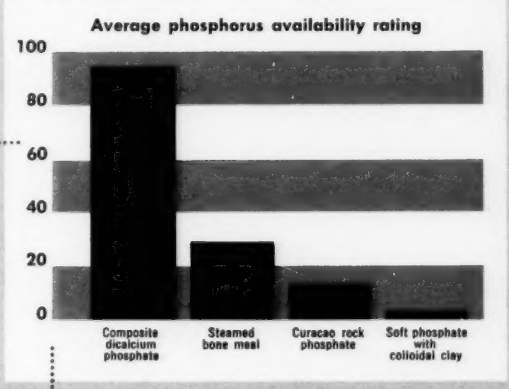
Oklahoma A & M work shows that "phantom" phosphorus from low-grade phosphates also penalizes cattle gains. In a 3-month feeding trial, calves fed a ration containing soft phosphate gained only 57 pounds. Calves fed dicalcium phosphate gained more than 2½ times as much — 145 lbs. — in the same period.

Pigs too make faster, more efficient gains when rations contain top-quality phosphorus. At Purdue, pigs fed soft phosphate averaged only .84 lb. of gain daily . . . required 2.72 lbs. of feed per lb. of gain. Pigs fed an equal amount of phosphorus from dicalcium phosphate averaged 1.38 lbs. daily — more than ½ lb. extra gain daily. And it took nearly ½ lb. less feed per lb. of gain.

Other research shows similar results. This big difference in phosphorus quality is one reason why many leading feed manufacturers are using superior quality dicalcium phosphates, such as International's dynamic Dynafos, in their livestock feeds. It's the economical way to insure complete phosphorus nutrition.

Tests prove the high biological availability of dicalcium phosphate

An *in vitro* fermentation study with rumen organisms provides further evidence that dicalcium phosphate rates much higher in available phosphorus than ordinary feed phosphorus supplements. Results of this Iowa State College study are shown in the bar graph.



All phosphorus supplements were compared to a standard sodium-potassium phosphate mixture.

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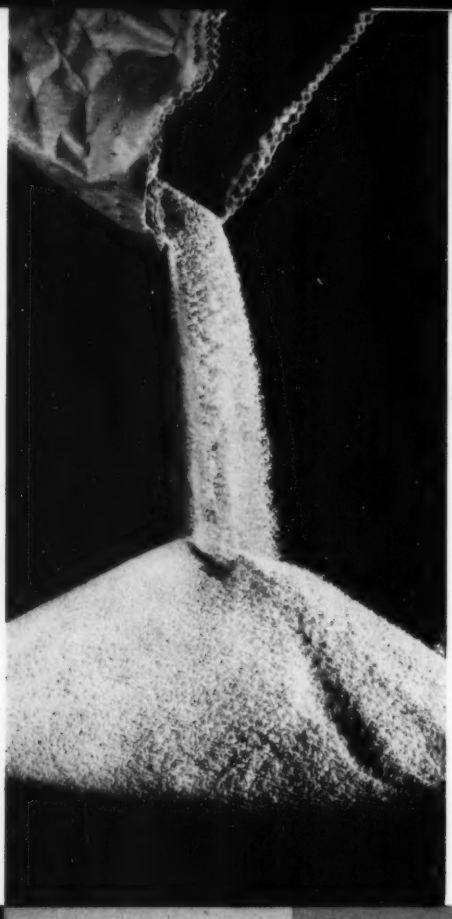

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DYNAFOS

is today's superior feed phosphorus source

Uniform beaded texture . . . dustless . . . free-flowing. Dynamic Beaded Dynafos — International's dicalcium phosphate — combines every advantage of an easy-to-handle product with nutritionally superior results. That's why so many leading feed manufacturers are helping farmers beat the high cost of "phantom" phosphorus with International's dynamic Dynafos.

Proven high biological availability assures more usable phosphorus in every pound . . . a harder-working dicalcium phosphate for modern feeds.

Get detailed data in this *Free* book

This FREE booklet documents the facts about "phantom" phosphorus. It includes the latest scientific developments in phosphorus nutrition . . . research reported by leading colleges and experiment stations. And it reviews International's own extensive research in all phases of phosphate nutrition and production. It's yours for the asking. Simply write: Technical Service Department . . .

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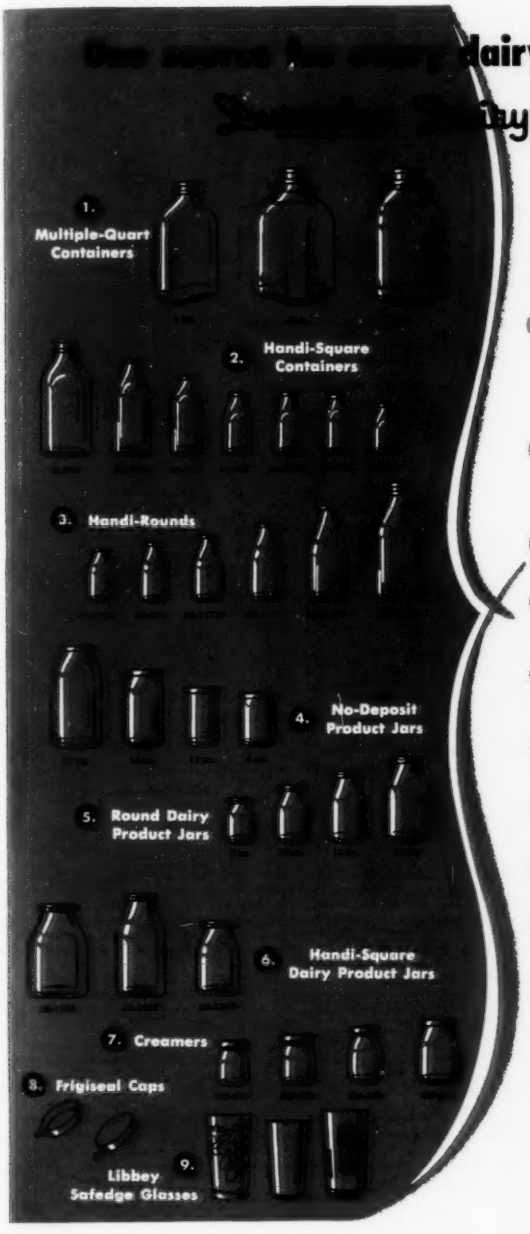

dairy container need . . .

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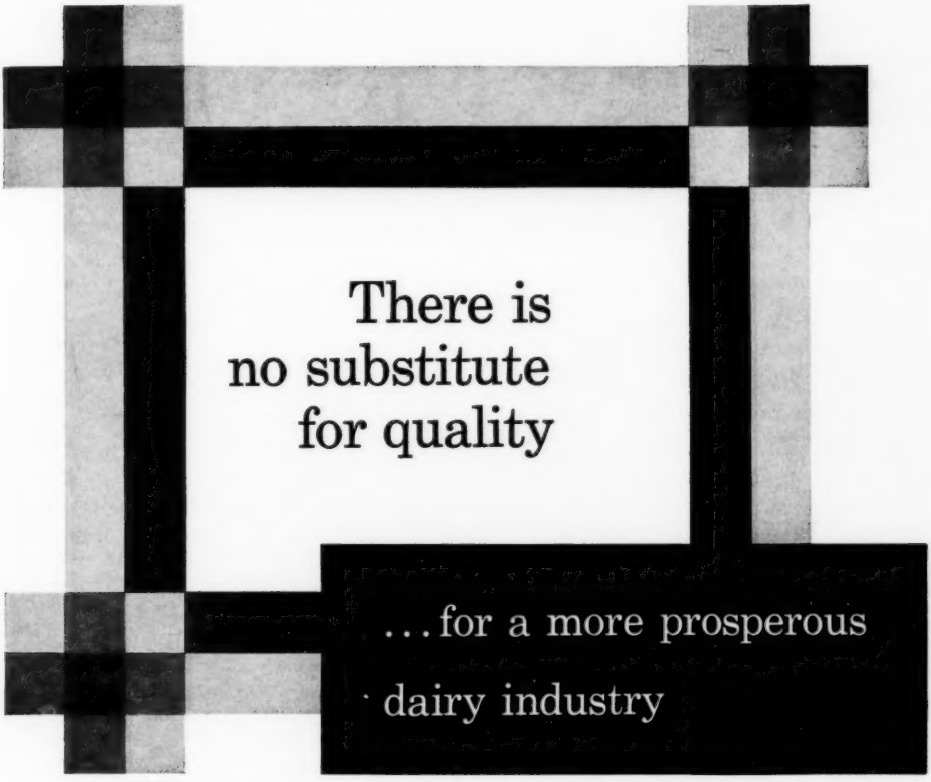

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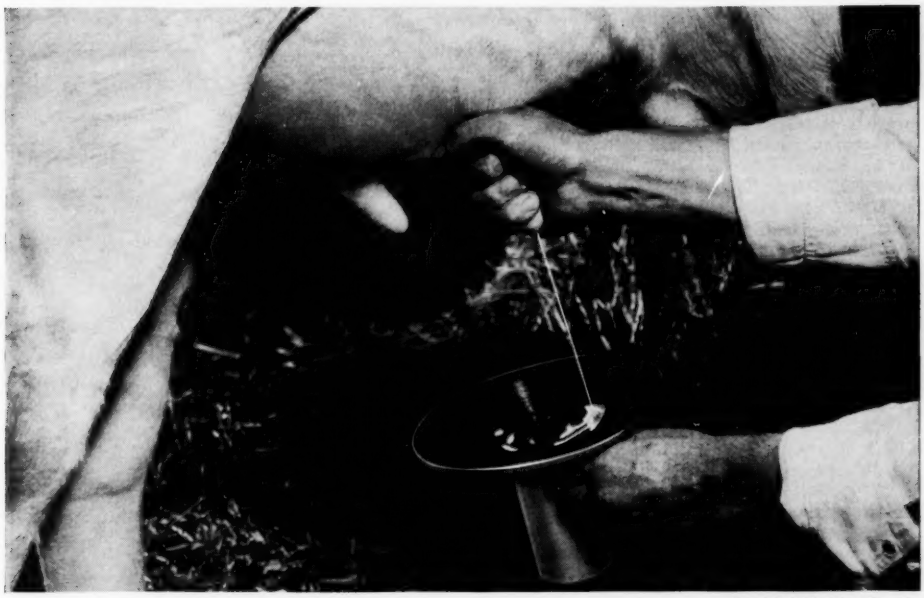
Dr. W. E. Petersen says:

"Generally MASTITIS

Produces Clots of Varying Sizes which can be Detected by Proper Use of the Strip Cup"



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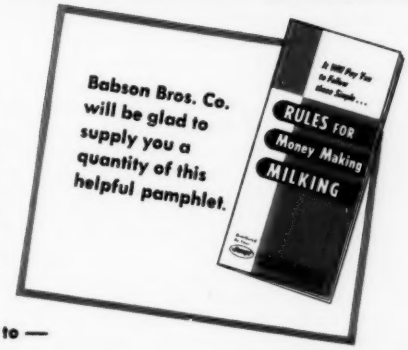

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Surge

PEOPLE and EVENTS
in the Dairy Science World

NDC Elects Judkins

H. F. JUDKINS has been named chairman of the National Dairy Council (NDC) dairywide coordinating committee for 1957. Mr. Judkins is the Secretary-Treasurer of the A. D. S. A., one of the 14 participating groups. He replaces LESTER J. WILL, former general manager of the American Dairy Association. For the position of vice chairman, the dairywide group named GAYLORD WHITLOCK, Director of Health Education at NDC. He replaces LLOYD H. GEIL, Director of Public Relations at NDC, who had held this position since the group was organized, in 1954.

The dairywide coordinating committee on nutrition research has selected as its major project in the coming months the problem of fat and its possible relationship to heart disease. It met at Chicago on March 18 to give further consideration to developing a program of action.

WENDELL H. GRIFFITH, Chairman, Department of Physiological Chemistry, Univ. of California Medical Center, Berkeley, met with the dairywide coordinating committee during its deliberations and addressed the group after its dinner meeting. He urged the dairy industry to appraise calmly the problem created by unfavorable publicity implicating dairy foods with coronary heart disease. In substance, Dr. Griffith said that a preponderance of evidence points to milk and its products as vitally important to human health. Any accumulating research evidence relating diet and heart disease must be and is being evaluated in the light of positive information already available.

The purpose of the Dairywide Coordinating Committee is to work toward better coordination of nutrition research in the dairy industry and greater use of nutrition facts by the dairy industry in its promotional and educational campaigns. Its five-point program, as established when the committee was organized three years ago, is to *(1)* assemble and evaluate from available sources the consumer and professional attitudes toward dairy foods; *(2)* review scientific data to provide the factual background for public information about dairy foods; *(3)* suggest additional needed nutrition research which will encompass all phases of human nutrition as affected by dairy foods; *(4)* present to the dairy industry authentic information about dairy foods, and to formulate recommendations for use in public relations, advertising, and other educational work; *(5)* make available to dairy groups an annual summary of dairy industry–sponsored nutrition research projects in progress, including the name of the director, the location of project, and the objectives.

The 14 members of the committee are: American Butter Institute, American Dairy Association, American Dairy Science Association, American Dry Milk Institute, American Farm Bureau Federation, Dairy Industries Supply Association, Evaporated Milk Association, International Association of Ice Cream Manufacturers, Milk Industry Foundation, National Cheese Institute, National Creameries Association, National Dairy Council, National Milk Producers Federation, and Purebred Dairy Cattle Association.

Dairy Products Improvement Institute Holds Annual Meeting

The Tenth Annual Meeting of the Dairy Products Improvement Institute was held Feb. 14, 1957, in New York City.

The Institute's president, A. C. FISHER, General Ice Cream Corporation, Schenectady, N. Y., presided during the main session in the afternoon, which was attended by 181 persons from industry, regulatory and educational fields, and the press.

Officers re-elected. A. C. FISHER, president; A. C. FAY, H. P. Hood and Sons, Boston, Massachusetts, vice-president; ROBERT C. HIBBEN, International Association of Ice Cream Manufacturers, Washington, D. C., treasurer; A. C. DAHLBERG, Cornell Univ., Ithaca, N. Y., secretary and adviser to the board. DONALD H. RACE will continue to serve as field director.

Directors elected to fill vacancies created through expiring terms and enlargement of the board are A. J. CLAXTON, Meadow Gold Dairies, Pittsburgh, Pa.; WALTER JUSTIN, Foremost Dairies, Inc., Philadelphia, Pa.; A. J. LUCAS, The Isaly Dairy Company, Pittsburgh, Pa.; RICHARD W. CHARLES, Supplee-Wills Jones Milk Co., Philadelphia, Pa.; E. J. ROBERTS, Crowley's Milk Co., Inc., Binghamton, N. Y.; and JAMES W. BELL, Blue Boy Dairy, Rochester, N. Y. W. A. WENTWORTH, Frankfort, Ky., retired president and director of the Institute, was unanimously re-elected honorary director.

Résumé of papers. WALTER TIEDEMAN, Executive Director, National Sanitation Foundation Testing Laboratories, Inc., School of Pub-

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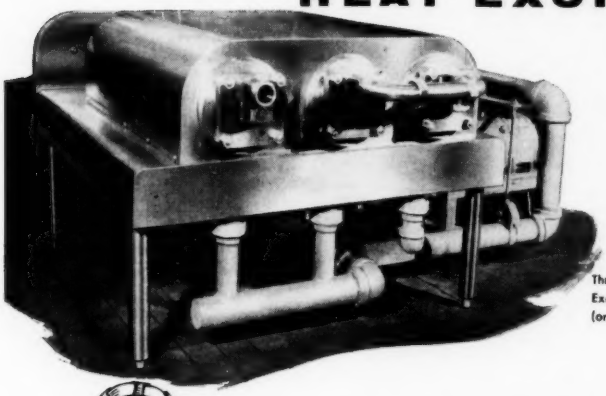

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lic Health, Univ. of Michigan, Ann Arbor, discussed "The Effect of Current Budget Pruning Trends on the Future Status of Official Sanitary Control of Milk and Milk Products." His conclusions are summarized as follows:

> It is quite certain that under the lash of budget control there will be an increasing tendency for health officials to saddle the milk industry with as much as possible of the responsibility for maintaining the safety as well as the quality of public milk supplies. More and more the public will look to the milk dealer for a warranty as to the quality and safety not only of milk products but of market milk. However, some well-directed official control of milk sanitation always will be necessary and, no doubt, will be available during the next 50 years or more, notwithstanding efforts to direct official activity elsewhere.

HAROLD B. ROBINSON, Chief, Milk Sanitation Section, Public Health Service, U. S. Dept. of Health, Education and Welfare, Washington, D. C., presented a paper on the history of the dating of pasteurized milk. He stated that the U. S. Public Health Service Standard Milk Ordinance and Code had not required dating since 1934. He concluded that the disadvantages far outweigh the advantages and that as a public health measure it is unnecessary.

ROBERT W. METZGER, Director of Quality Control, Dairymen's League Cooperative Association, Syracuse, N. Y., discussed the trends toward uniformity in methods of making inspections, particularly farm inspections in different localities. A proposed industry-farm-industry sanitation report form was presented which, if accepted by all government inspection agencies as proof of inspection by industry, would eliminate any confusion resulting from the use of more than one form, regardless of the number of agencies supervising a supply. The speaker felt that more and more attention should be given to odors and flavors of milk and milk products.

A. F. RANNEY, Animal Disease Eradication Branch, USDA, presented interesting statistics showing the tremendous strides that have been made in the eradication of brucellosis. In 1934, when the program was begun, slightly more than three million cattle were tested and of these almost half a million were reactors. During World War II there was some retrogression in the progress made. However, much has been accomplished since 1947. In 1955, total dollar losses due to the disease were reduced by approximately 50 per cent. There are six modified certified brucellosis-free states and 351 counties in 27 other states and Puerto Rico which have been so designated. Several states are fast approaching a statewide certified status.

DONALD H. RACE, Field Director, Dairy Products Improvement Institute, Ithaca, N. Y., discussed industry and regulatory application of the requirements for the sanitary production of sweet cream and milk for manufacturing.

International Dairy Show Scheduled for Oct. 9-15

The fifth annual International Dairy Show will be held Oct. 9-15 in Chicago's International Amphitheatre.

HILTON BOYNTON, Univ. of New Hampshire, Durham, will judge Ayrshires; Brown Swiss cattle will be judged by HARVEY SCHWARTZ, Waukesha, Wis.; GORDON CAIRNS, Dean of Agriculture, Univ. of Maryland, College Park, will judge Guernseys; Holstein-Friesians will be placed by GEORGE TRIMBERGER, dairy cattle specialist, Cornell Univ., Ithaca; Jerseys will be judged by DALE DEAN, Ridgeway, Mich.; and W. ARTHUR SIMPSON, Lyndonville, Vt., will judge in Milking Shorthorn competitions.

L. M. BROOKS, a prominent purebred Milking Shorthorn breeder and exhibitor, of Hope, R. I., has been elected a new director to the International Dairy Show board.

Shaw Attends Netherlands Meeting

J. C. SHAW, professor of dairy husbandry, Univ. of Maryland, College Park, was one of the U. S. representatives at a meeting on the uses of isotopes and radiation in agricultural research, held in Wageningen, The Netherlands.

The meeting was sponsored by the Food and Agriculture Organization of the United Nations. The other U. S. representative was P. B. PEARSON, chief of the division of biology and medicine of the U. S. Atomic Energy Commission.

Penn State U. Appoints Farrell V-P in Charge of Research

MICHAEL A. FARRELL has been appointed vice-president for research at Pennsylvania State Univ., University Park. He has served as director of the Agricultural Experiment Station since 1952.

As head of Penn State's agricultural research program, Dr. Farrell is responsible for more than 325 studies in all phases of agriculture, both on the main campus and at outlying field stations throughout the state.

He received his bachelor's degree from Penn State, his master's degree in bacteriology from Lehigh Univ., Bethlehem, Pa., and his doctor's degree in bacteriology from Yale Univ., New Haven, Conn.

Creamery Package Promotes Mitten

HORACE L. MITTEN, JR., Senior Research Engineer with Creamery Package Manufacturing Co., has been named Assistant to the Vice-President of that firm.

Mr. Mitten is a graduate of Ohio State Univ., Columbus, and received his M.S. in Agriculture Engineering at Michigan State Univ., East Lansing. Before joining Creamery Package, he was on the teaching and research staff of Ohio State.

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NDC Elects Directors

Fifty dairy leaders have been elected to the 1957 National Dairy Council (NDC) board of directors at the 42nd NDC Winter Conference–Annual Meeting held in Omaha, Neb.

Regional directors elected were: *Eastern Region*—F. B. BALDWIN, Abbotts Dairies, Inc., Philadelphia, Pa.; W. P. DAVIS, New England Milk Producers Assoc., Boston, Mass.; F. J. LAPEIRE, Waddington Milk Co., Maspeth, L. I., N. Y.; A. R. MARVEL, Inter-State Milk Producers Cooperative, Philadelphia, Pa.

Southern Region—J. M. FORBIS, Forest Hill Dairies, Inc., Memphis, Tenn.; ELFRETH JOHNSON, White Dairy Farm, Birmingham, Ala.; MOSE KISER, Guilford Dairy Cooperative Assoc., Greensboro, N. C.; P. E. REINHOLD, Foremost Dairies, Inc., Jacksonville, Fla.

Central Region—T. K. HAMILTON, Diamond Milk Products, Inc., Columbus, O.; J. A. MOSER, Louisville, Ky.; G. L. OGLE, Ideal Pure Milk Co., Inc., Evansville, Ind.; W. E. WINN, Pure Milk Assoc., Chicago, Ill.

Western Region—G. S. BULKLEY, Carnation Co., Los Angeles, Calif.; FRED DOYLE, Topeka, Kan.; W. C. MOORE, Denver Milk Producers, Inc., Denver, Colo.; R. H. REEVES, Carlson-Frink Co., Denver, Colo.

Ohio Dairy Prod. Assoc. Elects

RICHARD MCCELLAND, McCelland's Home Dairy, Lancaster, O., has been named President of the Ohio Dairy Products Assoc. Other new officers are Vice-President A. L. SHOUGH, Kroger Co., Dayton; Treasurer JOHN BURNS, Blue Valley Creamery, Columbus; and Treasurer Emeritus C. T. ALEXANDER, Columbus.

Templeton Takes Post in India

H. L. TEMPLETON, Fairmont Foods Co., Omaha, Neb., has accepted an appointment as Dairy Technology Advisor with the International Cooperation Administration. He will serve in India where he will aid in the development of the dairy industry of that country.

Dr. Prucha Dies in Florida

M. J. PRUCHA, Professor Emeritus of Dairy Science at the Univ. of Illinois, Urbana, died March 18 at his home at New Smyrna Beach, Fla. Dr. Prucha was 82 years old. Born in Bohemia, he served as Chief of Dairy Bacteriology at Illinois. An honorary member of the A.D.S.A., Dr. Prucha received his Ph.D. degree from Cornell Univ., Ithaca, N. Y. He is survived by his widow and three children.

Kansas News Items

The dairy program of the 89th Annual Farm and Home Week was held Feb. 4-6 at Kansas State College, Manhattan. Included were meetings of the State Breed Associations, annual meeting of the Kansas Interbreed Dairy Cattle Council, the annual Dairymen's Dinner, and the Dairy Day Program on problems of special interest to dairymen.

The 17th annual meeting of the Kansas Butter and Chese Institute was held in Wichita, Feb. 7. The program featured quality problems, and promotion of butter and cheese. Officers elected for the coming year were: president, H. P. REYNOLDS, DeCoursey Cream Company, Moline, Kansas; vice-president, G. C. FREEBY, Neosho Valley Co-op., Erie, Kansas; executive secretary, W. H. MARTIN, Dairy Husbandry Department, Kansas State College.

A bulk-tank drivers school will be held at the college March 22, in cooperation with the State Board of Agriculture and the State Board of Health. Subjects included are qualifications and responsibilities of drivers, sampling procedures, off-flavors, and judging practice. Regulatory and sanitary aspects of the bulk milk system also will be considered.

The 7th annual meeting of the Kansas Dairy Fieldmen's Association was held in Kansas City Feb. 21-22. A variety of topics relating to problems of fieldmen were on the program.

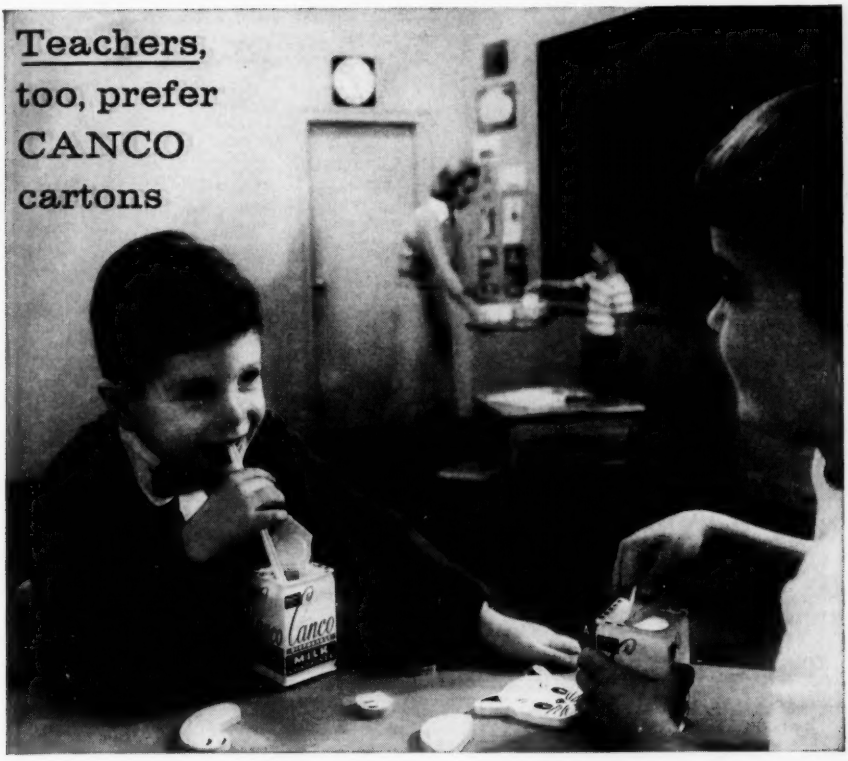
W. H. MARTIN of the Dairy Husbandry Department staff will participate in the program of the annual meeting of the Ontario Ice Cream Manufacturers' Association, at Niagara Falls, Ontario, Canada, March 19. He will attend also the Dairy Products Short Course at Iowa State College, Ames, on March 26, to discuss some aspects of ice cream manufacturing.

Ohio State News

About 450 people attended the 24th Annual Dairy Technology Conference at The Ohio State University, Feb. 5-8. Discussions by 39 visiting speakers dealt with problems in the fields of butter and cheese, field work, market milk and engineering, and ice cream and concentrated milk products.

The theme of the Conference was "Pathways to Opportunity." The banquet, sponsored by the Dept. of Dairy Technology and the four Ohio Dairy Technology Societies, was highlighted by an address by Dean L. L. RUMMELL of the College of Agriculture, who spoke on "Ohio State and the Far East."

About 45 volunteer workers for the Ohio State University Development Fund used the facilities of the Dept. of Dairy Technology, Feb. 23. Exhibits were arranged to demonstrate the progress achieved in the research areas supported by the Development Fund.



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The Dept. of Dairy Technology has recently released Vol. II of *Research in Review*, a report on the research activities in the department for the years 1954-1955.

The fourth annual Career Day for high school students will be held April 13. The program will include a tour of the Dept. of Dairy Technology facilities, and presentations by representatives of the College of Agriculture and the department on University procedures for enrollment, the scholarship program, and opportunities in the dairy industry. A luncheon will conclude the program.

The affair is sponsored jointly by the Maumee Valley, Northeastern Ohio, Central Ohio, and Cincinnati Dairy Technology Societies, and the Ohio Dairy Products Association in cooperation with the Dept. of Dairy Technology.

The Ohio Agricultural Experiment Station, Wooster, celebrated its Diamond Jubilee, March 1. The day was climaxed by a dinner with addresses by Gov. C. WILLIAM O'NEILL and BYRON SHAW, USDA.

The 40th annual convention of the Ohio Dairy Products Association was held Feb. 18-20. Financial problems and public relations were the main topics for discussion. Out-of-state speakers included R. HIBBEN, Association of Ice Cream Manufacturers, Washington, D. C.; C. HENTRICK, American Dairy Association, Chicago; P. ELLSWORTH, Milk Industry Foundation, Washington, D. C.; M. L. ORNER, A. E. Friedgen, Inc., New York City; V. MARTIN, Newton, Ia.; A. E. HALL, Brock-Hall Dairy, Hamden, Conn.; and N. MYRICK, American Milk Review, New York City.

During the convention the Education Committees of O. D. P. A. and Ohio Dairy Boosters Association met to discuss the role of the respective organizations in developing future leaders in the dairy industry.

Maryland News

WALTER F. WILLIAMS recently joined the staff of the Dairy Department at the Univ. of Maryland, College Park, where he will be conducting research work on the physiology of milk secretion and rumen function. Dr. Williams was recently associated with the Dairy Department at the Univ. of Missouri, Columbia, where he was conducting research in similar fields.

RICHARD L. WASHER has recently assumed a position in the Extension Service of the Univ. of Maryland and as Manager of the Maryland Artificial Breeding Cooperative. Mr. Washer earned his master's degree from Rutgers Univ., New Brunswick, N. J.

News from North Carolina

LESTER C. ULBERG joined the Dairy Husbandry Section of the Animal Industry Dept. on March 1, 1957, as a replacement for R. B. CASADY, who transferred to the Zoology Dept. Dr. Ulberg took his training with L. E. CASIDA at the Univ. of Wisconsin, Madison, in physiology of reproduction. After two years as an instructor at Wisconsin, he moved to Mississippi State College, State College, in the Animal Husbandry Dept. where he worked on reproductive problems of sheep and beef cattle. In addition to teaching undergraduates and directing the graduate students, he will conduct research on environmental effects on reproduction in both male and female dairy animals using temperature and humidity control chambers.

Economic Outlook for Dairying in Minnesota

E. FRED KOLLER, agricultural economist at the Univ. of Minnesota, St. Paul, reports that there are about 30 per cent less Minnesota farmers selling milk or cream now than in 1939. But during the same period, individual herds have been getting bigger and fewer; and total milk production has increased slightly.

Dairy plant trends tell a similar story. In 1935 there were 875 butter-making plants in Minnesota, compared to 561 in 1956. The change to larger volume, both on the farm and in the dairy plant, is the result of normal economic adjustment to increased costs and decreased profit margins, Koller explained.

There are 20 per cent less dairy cows in Minnesota now than in 1944, but average milk output per cow has increased about 100 pounds every year. In 1955, the average Minnesota cow produced 6,380 pounds. Average milk output per farm has increased 35 per cent. This increase is due to bigger herds, better breeding, and better feeding and management.

At the same time, farmers are stepping up their equipment investments. Farmers who use bulk milk tanks instead of 10-gallon milk cans now produce 17 per cent of the total volume of milk marketed in Minnesota. Milking parlors and pipeline milkers are finding their way to more farms. In order to reduce the cost per unit of these investments, farmers have found it necessary to increase their milk volume.

Milk is going to the plant in a different form from that of 20 years ago, too. From 1935-39, 85 per cent of Minnesota's milk was sold as farm-separated cream. Today, only 30 per cent is sold as cream and the remainder is whole milk. Koller says the proportion of whole milk sold in Minnesota may be even higher in a few years.

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M.S. Degree

MARVIN P. THOMPSON—Viscosity of skimmilk as affected by ion concentration. Kansas State College, Manhattan.

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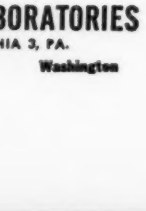

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California IFT Elects Officers

The Institute of Food Technologists, Southern California Section, has elected the following officers: chairman, E. B. OBERG, Director of Research, Carnation Co.; vice chairman, G. C. BEISEL, Head, Development Department, Exchange Orange Products; treasurer, S. I. DULKIN, Co-Director, Chem Tech Laboratories; and secretary, GORDON E. MILLER, Technical Representative, C & H Sugar Refining Corporation, Ltd.

Musser Retires

KARL B. MUSSER, after 37 years of service, has retired from The American Guernsey Cattle Club. He was secretary-treasurer of the organization 31 years, and for the past two years was executive director–treasurer. He will continue to serve on the club's research committee and is currently secretary of the Purebred Dairy Cattle Association.

ROBERT D. STEWART, who was appointed secretary two years ago, now is secretary-treasurer.

USDA Reports Increases in Exports

Nonfat dry milk exports, exclusive of donations to private relief agencies, increased from 231 million pounds in 1955 to a record 307 million pounds in 1956, the USDA reported recently. Largest gains were in shipments to the Far East, with Japan and Korea each taking more than 50 million pounds. Large increases in exports in the past three years reflected the influence of government programs and sales to foreign governments at concessional prices.

Dairy Sales for Export Reported

Disposals of government-held dairy products reported on this month by the USDA included sales for government export of 315,538 pounds of butter at prices ranging from 36.9 to 39 cents per pound; 1.6 million pounds of Cheddar cheese at 22 cents per pound; one million pounds of nonfat dry milk at 7.55 to 9.9 cents per pound, and 161,894 pounds of butter for domestic use in extending cocoa butter at 37.58 to 39 cents per pound. On March 5, USDA announced that purchase authorization to India for purchase of nonfat dry milk under Title I, P. L. 480 was increased to $798,000 worth.

New Zealand Dairy Leader Finds U. S. Pressure High

Conditions in New Zealand do not create quite the pressure in agriculture that exists in the United States, according to A. P. O'SHEA, secretary of Federated Farmers of New Zealand. Mr. O'Shea was a recent visitor with the executive committee of the National Milk Producers Federation.

In dairying he predicted a bright future and a shortage of animal products in the United States by 1975. He stated that because of lower selling prices for New Zealand, their operations through necessity, were more streamlined and efficient. He urged cooperative dairy producers of New Zealand and U. S. to work together, and suggested that a delegation of American dairy farmers visit his country for the exchange of ideas.

Price-Support Activity for February Reported

Price support activity in butter and nonfat dry milk in February was lower than the comparable period a year ago, the USDA has reported. Butter purchases dropped 24 per cent and nonfat dry milk 46 per cent from February, 1956. Cheese purchases continued to increase and were 11 per cent over February last year.

During the 1956-1957 marketing year 131 million pounds of butter, 244 million pounds of cheese and 686 million pounds of nonfat dry milk were utilized in various government programs.

Maryland-Delaware Ayrshire Breeders Elect Officers

The following officers of the Maryland-Delaware Ayrshire Breeders' Association have been elected: R. W. TRACY, Parkton, Md., president; C. BROOKS NAGEL, Preston, Md., first vice-president; L. D. CAULK, Wyoming, Del., second vice-president; and ALLEN HILL, Woodbine, Md., secretary-treasurer. Directors elected were PHILIP KNATZ, Owings Mills, Md.; JOHN SHANK, Hagerstown, Md.; and HOWARD W. CLARK, Glenelg, Md.

Eleven breeders received Longtime Production Certificates for 44 cows that so far have produced a total of 2,000 pounds or more of butterfat. They are WILLIAM ATTIX, Dover, Del.; HOWARD W. CLARK, Glenelg, Md.; William H. Hill and Sons, Woodbine, Md.; PHILIP KNATZ, Owings Mills, Md.; C. G. Linthicum and Sons, Boyds, Md.; WILLIAM A. MOORE, Mt. Savage, Md.; JOHN H. NICOLAI, Ellicott City, Md.; EDWARD C. WILSON, Newark, Del.; JOSEPH M. ZOLLER, JR., Ellicott City, Md.; Draper Food, Inc., Milford, Del.; and WALTER J. HAHN, Frederick, Md.

E. O. Dame Joins DeLaval

E. O. DAME has sold his dairy equipment and service business and accepted a position as field engineer for DeLaval Separator Co., Poughkeepsie, N. Y., to do engineering and equipment installation supervision in the eastern part of the U. S.

Borden's Names New Credit Manager

EDWARD H. BULGER has been named manager of the Borden Company's Credit Dept. Formerly assistant manager, he succeeds HERBERT C. LUHRS who retires after 47 years of service. Mr. Bulger started with the department 35 years ago as a clerk. FRED M. GRAU, a member of the department for 38 years, moves up to the assistant manager's post. At the same time, EDWARD F. GEOTZ has been named office manager of the department.

Marketing Research Unit Established at Borden Company

A new marketing research unit designed to serve all segments of the company has been established at the Borden Company, New York City.

The unit has been formed in recognition of the growing importance in modern marketing of the use of consumer and trade research in the development and merchandising of new products. It further requires specialized experience and training which may not be available in the sales and advertising departments of Borden's decentralized operating divisions, or in their advertising agencies.

ROBERT E. KAHL, national advertising manager in charge of merchandising and research, will manage the new staff service. Mr. Kahl joined Borden's in 1952 after three years with Young & Rubicam, New York City, as research supervisor on the Borden account. Before that he gained marketing and merchandising experience with the U. S. Department of Agriculture, the Kroger Company, and—during the war—the Office of the Quartermaster General of the U. S. Army.

Where to Send It

Throughout the year many members and subscribers may have occasion to correspond with the Association about subscriptions to *The Journal of Dairy Science.*

So that all inquiries may be given prompt attention and quick action, the Association requests that you make a note of this brief outline of "where to send it."

Membership correspondence (including material relative to members' subscriptions to the Journal) — The American Dairy Science Association, c/o H. F. JUDKINS, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, N.Y.

Nonmember subscription correspondence — The Journal of Dairy Science, c/o The Garrard Press, 119-123 West Park Avenue, Champaign, Illinois.

STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

E. L. THOMAS, *Editor*

Connecticut Dairy Club News

The Connecticut Dairy Club activities for the current year started with the annual supper and social evening. An additional activity of the 40 members of the Club was the sponsorship of the annual dairy cattle and products judging contest, open to all interested students at the Univ. of Connecticut. Members of the intercollegiate judging teams served as official judges. F. CAPELL won the dairy products judging prize, as well as the sweepstakes prize, and C. ALYNN received the cattle judging prize.

Subjects presented at the club meetings during the past semester have included: "Communications in the Dairy Industry," by F. ATWOOD, Farm Editor of Radio Station WTIC, Hartford; "The Organization and Function of the Dairy Division," State Department of Agriculture, by S. MORSE, Senior State Milk Inspector; "Forage Production," by E. LOEW, New England Green Pasture Winner; "Getting Started in the Dairy Business," by C. KNUDSEN, manager of Knudsen's Dairy; and "Freezing Semen," by A. BARISONE, commercial semen processor.

With spring approaching, arrangements are being made for the annual Dairy Club Breakfast and Fitting and Showing Contest on May 5. The highlight of the breakfast will be the annual Honor Awards to two outstanding dairy leaders in the state—one in the field of dairy production and the other in dairy manufacturing. The breakfast has become very popular, drawing alumni and friends in the dairy industry from all parts of Connecticut and neighboring states. In the afternoon, students compete for prizes in the fitting and showing contest, which is interspersed with student-faculty milking and can-rolling contests.

Kentucky Club Sponsors Showmanship Contest

The Univ. of Kentucky Dairy Science Club, Lexington, held its annual Showmanship Contest Jan. 17 at the Dairy Center at the University. The contest was divided into two classes according to breed—Holstein and Jersey. The heifers shown were allocated to the contestant by lot. There were 14 contestants in the show judged by Mr. TED HOWARD of the Univ. of Kentucky staff.

In the Jersey class, RANDALL BARNETT, Senior, was decided the winner, and SMITH MITCHELL, senior and president of the Dairy Club, was winner of the Holstein class. Miss JOYCE HILL SMITH, Kentucky Dairy Princess, presented winners with their awards.

In placing for Grand Champion, Mitchell was chosen the winner and his name will be engraved on a plaque which will hang in the Dairy Products building. Both contestants received trophies donated by the American Jersey Cattle Club and Holstein-Friesian Association of America.

A milkmaid contest, a water-filled balloon pitch-and-catch contest, and stories by the Master of Ceremonies, ALBERT WILSON, senior, provided entertainment.

Review of Maryland Club Activities

The Dairy Science Club of the Univ. of Maryland, College Park, had its first meeting for the academic year of 1956-57 in September, and elected the following officers: president, CARL WINFIELD, JR.; vice-president, PERRY JOHNSTON; secretary, JOHN WARFIELD, treasurer, JAMES STEWART.

At this meeting, the club voted to meet on the second and fourth Tuesdays of each month, instead of having the usual monthly meeting of previous years. This action was taken to strengthen the unity of the club through increased attendance, interest, and activities.

In October, the Club successfully initiated and completed its first project. The Dairy Science Club prepared and served 400 box-lunches to the members of the Maryland Artificial Breeders' Cooperative attending the Annual Breeders' Conference on campus. This project was an example of the policy of the Club, to participate in a service capacity at all functions of the Dairy Dept.

VERNON DOYLE, closely associated with producer cooperatives and marketing orders for many years, addressed the October meeting. A thorough knowledge of the purpose and operation of a marketing order was obtained by all in attendance.

During the October meeting, the Club voted to establish a permanent record of its recognition to the outstanding judges on the Dairy Cattle and Dairy Products Judging Teams each year. It was decided that a permanent plaque be purchased and presented to the Dairy Dept., to which the appropriate names can be added each year. The club will also present a small trophy, properly engraved, to the outstanding member of each team annually.

In November, the Club duplicated the box-lunch service at the Annual Dairy Technology Conference. The box-lunches were identical to those furnished the producers' group and consisted of two sandwiches, potato chips, pie,

ice cream, and milk. Again the service was highly accepted and successful.

The speaker at the November meeting was ALLAN DAY, who recently joined the Dairy staff. Dr. Day did his undergraduate work at the Univ. of Maryland and held the position of president of the Dairy Science Club in his senior year. He discussed radiation sterilization as applied to agriculture.

WALTER C. ENGLAND, England Laboratories of Washington, D. C., presented an excellent collection of slides on the dairy industry in Holland to the group at the December meeting.

Minnesota Dairy Science Club News

The Univ. of Minnesota Dairy Science Club sponsored a "Milk Hour" at the Student Union on November 20. Princess Kay of the Milky Way, BARBARA JEAN BOSSUS, served as hostess. Milk, cheese, and crackers were served to acquaint students with the wholesome, fine flavor of dairy products.

Farm and Home Week, an annual event, was held Jan. 7-11. This is a week in which short courses are offered in all phases of farming and homemaking to interested residents of the state. The Dairy Science Club served dinner on Jan. 9 and 10 in an effort to raise funds for club expenses.

The dairy portion of the Winter Judging Contests was held on February 2. These contests were open to all interested students on campus. Pen-and-pencil sets were awarded to winners of individual contests, and a trophy was awarded to the top individual participating in both dairy products and dairy cattle judging. Winners of all judging contests were announced at the annual Ag. Stag Banquet on Feb. 14.

At its Feb. 5 meeting, LES WILSON, secretary of the Minnesota Guernsey Breeders Association, told of his experiences in the selection and classification of dairy cattle.

Rhode Island Dairy Students Honored

LEO W. MINISCE of Narragansett, R. I., was awarded a Ralston Purina Scholarship on the basis of high scholarship, leadership, character, and citizenship potential. Mr. Minisce also was chancellor of Alpha Zeta and a member of both the University dairy cattle and dairy products judging teams. He hopes to enter a veterinary college next September.

LEON P. PIASCIK, West Warwick, and RALPH M. SHEFFLER, Rumford, were both awarded CHARLES H. HOOD Dairy Foundation awards

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for diligent effort and outstanding scholastic achievement, while majoring in Animal and Dairy Husbandry at this University. Mr. Piascik is in the junior class, while Mr. Sheffler is a senior and will be graduated this June.

HERBERT H. ROBERTS, Peace Dale, was awarded the CHARLES M. COX Trust Fund Scholarship, which is given on the basis of the student's need, character, and scholarship ability. Mr. Roberts will graduate this June with a major in animal husbandry and plans to go to graduate school.

ADOLF G. HELGERSON of Rumford was awarded the Virginia Dare Award, which is given to a senior with high scholastic average majoring in Dairy Husbandry, with a special interest in quality flavors for the commercial production of ice cream. Mr. Helgerson will graduate this June and plans to go into commercial work.

Virginia Tech Dairy Science Club Activities

The Virginia Tech Dairy Science Club has recently finished its first money-making project for the school year 1956-57. The club sold ready-packaged cheese for Christmas gifts. Over $700 worth of this cheese was sold, with a profit of about $140 for the club.

During the fall quarter, a series of programs on "Opportunities for College Graduates in Dairying" was held at the regular meetings. Included in this series were talks on research, teaching, extension, dairy plant management, and farming.

It was recently announced that DONALD SOWDER, a sophomore from Rocky Mount, was this year's recipient of the National Dairy Products Scholarship. This scholarship, valued at $150, is awarded annually to a sophomore on the basis of need, 4-H club work, and scholarship during his freshman year.

PAUL COX, Indian Valley, was named this year's recipient of the Virginia Dare Award. This award is made annually to an outstanding senior in dairy technology, by the Virginia Dare Extract Company.

The club is starting to make plans for the annual Dairy Day fitting and showmanship contest to be held April 27, in conjunction with the fourth V. P. I. Agricultural Exposition. PAUL KIPPS, last year's reserve champion, is in charge of this year's show. The students have about two weeks to fit the animals and train them.

Babcock Dairy Science Club Conducts Survey

At the December meeting of the Babcock Dairy Science Club, KEN RUNLEE and DAN MASON, who operate Dairyland Vendors of Madison, spoke on the problems and some of the techniques involved in selling milk and ice cream by the use of vending machines. Both Runlee and Mason are recent graduates of the Dept. of Dairy and Food Industries, and were active members of the Babcock Dairy Science Club. The talk was very practical and gave the club members a good chance to find out about this aspect of the dairy business.

A questionnaire has been sent out to get information from leading dairy manufacturers, dairy supply houses, and others connected with the industry, to help dairy students here find out what potential employers desire in graduates, and what is lacking, as they see it. This will also be a survey of job opportunities to acquaint students with companies in the dairy field. Preparations are being made, based on results of this questionnaire, to arrange for interviews between prospective employers and future graduates at the forthcoming Dairy Manufacturers' Conference in April.

LETTER TO THE EDITOR

On Reporting Institute, Conference Papers in the Journal

Many of the Dairy Departments throughout the country have annual conferences and institutes dealing with dairy manufacturing and dairy production and, at these meetings, outstanding dairy leaders present papers of great importance. Many of these valuable presentations are lost to the general public, because they are not published at all, or appear in abbreviated form in publications of limited circulation. Therefore, it would appear that these conferences and special institutes constitute a fertile field for papers worthy of publication in the "Our Industry Today" section of the Journal.

Would it not be in order for the various Dairy Departments to obtain copies of the better papers presented at these various meetings and to forward them as promptly as possible to the Editor of the Journal of Dairy Science? This not only would serve to keep the entire Industry advised of newer developments which may be revealed at these meetings but would give recognition to the authors and to the educational institutions involved. Could this not be a rather "painless" way of making a real contribution to the Journal and to the Industry?

I. A. GOULD, *Chairman*
Dept. of Dairy Technology
The Ohio State Univ., Columbus

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ACTIVATION OF BOVINE SPERMATOZOA BY THE USE OF SODIUM CARBONATE[1, 2, 3]

H. E. RICKARD, T. M. LUDWICK, E. A. HESS, AND F. ELY
Department of Dairy Science, The Ohio State University, Columbus, and Ohio Agricultural Experiment Station, Wooster

Environmental factors affect the livability of semen. Better results were obtained by storing semen at 37° to 39° C. at pH 7.1 to 7.4 than at pH 6.5 to 6.8. However, at 4° to 6° C., the lower pH values gave the best results. The semen was activated with sodium carbonate. *Editor.*

Although good conception has resulted from procedures now in effect in artificial breeding, there is some possibility that even better results might be obtained if more information were available concerning the regulation of spermatozoan motility and its relation to conception. In general, two approaches have been used to study this problem: *(a)* the influence of various levels of pH, and *(b)* the effects of added stimulants.

Freshly collected semen usually has a pH of approximately 6.8, and most common diluents are buffered accordingly. Somewhat greater sperm activity may be observed, however, for short periods of time if the pH is increased. Romijn *(9, 10)* reported that optimum sperm activity could be obtained at a pH of 7.2. In many cases, however, this alkaline medium appeared to have some detrimental effects on the duration of life of the spermatozoa. Lardy and associates *(8)* found a similar condition in ram semen. It was observed by Dubinick *(1)* that motility of spermatozoa ceased at a pH of 4.2, but after a shift to alkalinity, sperm motility was resumed. At a pH of 3.4 an irreversible reaction occurred which prevented the sperm from regaining motility even when the pH was raised. When epididymal samples were suspended in Ringers-glucose solution, Henle and Zittle *(3)* found that maximum respiration and motility were obtained with a pH of 7.5 to 8.0.

Many products have been added to semen samples in an effort to improve livability or motility. Schultze and Davis *(11, 12)* reported increased oxygen consumption and also increased conception rates when thyroxine was added to diluted semen. When Schultze and Mahler *(13)* added low concentrations of sodium arsenite to semen samples, respiration rates were increased, but Lardy

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[1] Contribution to North Central Regional Project NC-2, Improvement of Dairy Cattle Through Breeding.

[2] The material herein reported was taken from data submitted by the senior author in partial fulfillment of the requirements for the degree of Master of Science, The Ohio State University. 1953.

[3] The study was made possible through the assistance of Central Ohio Breeding Association, Richard Kellogg, Manager.

and Phillips *(7)* observed that iodoacetic acid definitely inhibited sperm motility. Glycolysis and respiration rates were stimulated when extracts from semen *(2)* or sodium bicarbonate *(6)* were added to epididymal samples. When low concentrations of sodium salts were added to diluted bovine semen samples, Kampschmidt and associates *(5)* observed increased survival rates.

Kaeser and Ludwick *(4)* added various amounts of sodium carbonate to diluted semen and obtained increased motility. It was observed, however, that optimum motility was somewhat dependent on storage temperature.

The chief objective of the work reported here was to determine the effects on sperm motility and fertilizing capacity when activated by sodium carbonate.

EXPERIMENTAL PROCEDURE

Semen used in this experiment was made available by Central Ohio Breeding Association. This semen was diluted immediately after collection with an egg yolk-citrate diluter (3.6% sodium citrate, 17% egg yolk plus streptomycin and penicillin).

Laboratory Test

Diluted samples were divided into four equal portions. The four portions were adjusted to different pH values by using ½% solution of sodium carbonate to raise the pH and 1% citric acid to lower it. The adjusted readings of the four portions were 6.5, 6.8, 7.1, and 7.4. The pH determinations were made with a Beckman glass electrode pH meter.

By using the split sample technique, the four portions were each divided, again thereby making two sets, four portions to each set. One set was refrigerated at 4° C. to 6° C. and the other set was incubated at 37° C. to 39° C.

An initial evaluation of the semen was made immediately after adjustment of the pH. At given time intervals, evaluations were made for progressive motility and the percentage of live spermatozoa (evaluation range 0–100). The samples under incubation were examined at 4, 8, and 12 hours from the time of pH adjustments, and the refrigerated samples were evaluated at 4, 6, 8, and 10 days from the time of pH adjustment.

Fertility Trial

After careful study of the laboratory results, a fertility trial was designed and carried out with the cooperation of the Central Ohio Breeding Association (August 21–October 3, 1953).

Eight technicians were selected to do the insemination of the cows for this trial. These technicians were chosen on the basis of their willingness to cooperate, as indicated by personal interview, and the number of cows they were expected to breed during the trial. Only Guernsey and Holstein sires were used since these two breeds account for a very high percentage of all cows bred.

At the time of collection, two full vials of diluted semen from each of the Holstein and Guernsey bulls collected were placed in the shipping box of each cooperating technician. Also placed in each shipping box were two vials, per

each Holstein and Guernsey bull collected, containing 0.15 ml. of ½% solution of sodium carbonate. On the vials containing sodium carbonate a mark was drawn at the 3.15-ml. level.

The semen was shipped from the bull stud immediately after collection and processing. All technicians received the semen the following morning. Thus, the semen was approximately 16 hours old at the time it was received by the technicians.

Procedure for first day. Prior to breeding the first cow, semen for experimental use was activated by pouring a portion of semen from one of the full vials into a vial containing the sodium carbonate solution. The vial of diluted semen was inverted slowly several times to help equalize the sperm concentration before activation. The vial containing sodium carbonate was filled with semen to the mark at the 3.15-ml. level. Semen in the vial containing sodium carbonate was the experimental sample and the semen which remained in the vial containing no sodium carbonate was the control.

Only first services were used in this trial. Each technician was instructed to breed alternate cows to the experimental sample and the remaining cows to the control sample. At the end of the day any of the experimental semen not used was discarded.

Procedure for second day. Prior to breeding the first cow on the second day after collection, 3 ml. of semen from the remaining full vial was poured into the remaining vial containing sodium carbonate. The procedure for breeding of the cows on the second day was the same as the first day.

All breeding receipts for the cows bred with the activated semen were marked by the technician at the time of breeding so that they could be separated from those of the control services.

RESULTS AND DISCUSSION

Laboratory Results

The means of the evaluation of 16 semen samples stored at four different pH levels at refrigerator temperatures (4° C. to 6° C.) are presented in Table 1. These are microscopic evaluations based on the percentage of live cells and motility rate (evaluation range—0 to 100).

TABLE 1

Means of evaluations of 16 samples for motility and per cent alive (Refrigerated at 4° to 6° C.)

Age of semen	pH Values							
	6.5		6.8		7.1		7.4	
	Mot.	*% A.*	*Mot.*	*% A.*	*Mot.*	*% A.*	*Mot.*	*% A.*
Initial	56.9	50.0	65.6	54.4	79.4	58.6	85.6	61.9
4 days	44.7	38.7	36.0	34.0	19.3	21.3	14.0	14.0
6 days	33.1	33.6	22.5	22.5	08.1	10.0	05.0	05.0
8 days	19.3	21.3	13.3	16.7	06.0	04.7	04.0	03.0
10 days	08.6	10.0	0.64	07.1	02.1	02.9	00.7	00.8

Microscopic evaluations tend to indicate that initial readings are improved as pH values vary from 6.5 to 7.4. The initial readings for samples adjusted to a pH of 6.5 are 56.9 and 50.0, and readings for the same samples adjusted to 7.4 are 85.6 and 61.9. This same trend does not continue, however, as semen is stored for longer periods of time. Readings would tend to indicate that semen can maintain better livability and motility at 4° C. to 6° C. when adjusted to a relatively low pH (6.5) compared to a high value (7.4).

In Table 2 are shown the microscopic evaluations for 16 semen samples stored at 38° C. These evaluations indicate that semen stored at incubation temperature will maintain better motility and livability at a high pH (7.4) than at a low pH (6.5).

TABLE 2

Means of evaluations of 16 samples for motility and per cent alive (Incubated at 37° to 39° C.)

Age of semen	pH Values							
	6.5		6.8		7.1		7.4	
	Mot.	*% A.*	*Mot.*	*% A.*	*Mot.*	*% A.*	*Mot.*	*% A.*
Initial	56.9	50.0	65.6	54.4	79.4	58.6	85.6	61.9
4 hr.	45.0	44.4	50.1	46.3	66.9	51.2	67.5	51.9
8 hr.	17.5	23.8	25.6	33.8	35.6	32.5	36.3	33.1
12 hr.	01.3	01.3	04.7	04.7	08.0	06.7	04.7	04.0

Evaluations of the trends in Tables 1 and 2 would indicate a possibility of increasing motility by increasing the pH of the semen sample when held at warm temperatures (38° C.). Since this procedure of activating the sperm could be easily duplicated in most artificial breeding organizations, a fertility trial was planned in which normally diluted semen with a pH of approximately 6.8 was stored at a temperature of about 6° C. and activated with sodium carbonate prior to insemination. The environment of the inseminated material would be very close to 38° C.

Fertility Results

The results of the fertility trial, which are presented in Table 3, are measured by % 60- to 90-day nonreturns to first services. Since the volume of activated semen was rather limited, the number of animals bred in the control group is considerably larger. With the use of 1-day-old activated semen, a

TABLE 3

A comparison of nonreturn rates between activated and control semen (1st service, 60–90 day nonreturn)

	Cows bred		% Nonreturn	
	Control	Activated	Control	Activated
1-day-old	547	366	72.02	72.68
2-day-old**	595	300	60.34	70.33
Total*	1142	666	65.94	71.62

* Significant at the 5% level.
** Significant at the 1% level.

difference of 0.66% in the nonreturn rate was shown above that of the control. Two-day-old activated semen had a nonreturn rate of 70.33% compared with a 60.34% for the control. Seven of the eight technicians had a higher rate with the use of 2-day-old activated semen compared with the 2-day-old control semen. When services from both 1- and 2-day-old semen were considered, all technicians had a higher nonreturn rate with activated semen than with the control.

A 2×2 table with a chi-square test was applied to the fertility trial data There was a significant difference at the 5% level between the nonreturn rates of the control and the activated semen. The same test was applied to the nonreturn rates of 1-day-old semen, but no significant differences were found. However, when the same test was applied to the nonreturn rate of 2-day-old semen there was a significant difference (0.01 level) between the activated and control semen.

The difference in conception between 1- and 2-day-old control samples was somewhat greater than expected; however, a major portion of this difference may be due to season. Results from this laboratory have indicated considerable spread between 1- and 2-day-old samples from winter to summer. A difference of only 2% has been observed in some winter months and as much as 12% difference during extremely hot months.

Although the above data include only results obtained with liquid semen, it may soon become practical to adjust the procedure to accommodate frozen semen.

SUMMARY

A study was designed and conducted to show some of the effects of various levels of pH on semen quality when held at different temperatures.

Microscopic evaluation of semen samples incubated at 37° to 39° C. indicated somewhat better livability at pH 7.1 to 7.4 than at pH 6.5 to 6.8. A reversed trend was observed when samples were stored at 4° to 6° C. Evaluation of these refrigerated samples indicated somewhat better livability at lower pH values (6.5 to 6.8 compared with 7.1 to 7.4).

A fertility trial was conducted with semen that had been activated by the addition of sodium carbonate. In this trial 666 cows were bred with the activated semen; 1,142 cows were bred with the control semen. With the use of 1-day-old activated semen the 60- to 90-day nonreturn rate to first services was 72.68% compared with 72.02% for the control semen. When 2-day-old activated semen was used, the nonreturn rate was 70.33% compared with 60.34% for the control. The nonreturn rate of all services with activated semen was 71.62% and the nonreturn of all control services was 65.94%.

Work is under way at the present time to develop a procedure for activation of frozen semen.

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ESTIMATING THE THYROID ACTIVITY OF DAIRY HEIFERS[1]

J. R. LODGE,[2] R. C. LEWIS, AND E. P. REINEKE
Departments of Dairy and Physiology
Michigan State University, East Lansing

Thyroid secretion in calves was measured with radioactive iodine, injected subcutaneously. There were individual and breed differences in iodine assimilation, the average being 41.6%. The calves were able to recycle iodine through the thyroid. *Editor.*

In 1952, Henneman *et al. (4)* reported the development of a method for quantitatively measuring the thyroid secretion rate of intact individual sheep. The procedure introduced by these authors involves direct counts of radioactivity in the thyroid region to determine the amount of thyroxine that must be given daily to suppress the output of previously collected I^{131} from the gland. Because of the methods of computation employed, this has been referred to as the extrapolation technique. The method was confirmed and extended by experiments in rats by Reineke and Singh *(10)*. It has been used by Henneman *et al. (5)* and Singh *et al. (11)* to study some of the environmental and physiological factors related to thyroid function in sheep.

A method reported by Pipes and Ruppert *(8)* and Pipes and Turner *(9)* differed from the above in that radioactivity counts were taken on samples of blood plasma instead of directly over the thyroid. It has the disadvantage of being considerably more tedious to apply. Administration of sufficient quantities of I^{131} to insure satisfactory counting levels in blood plasma also introduces serious danger of thyroid injury in the animals through excessive radiation.

Preliminary reports on the use of the extrapolation technique in dairy calves were given by the present authors in 1955 *(7)* and again in 1956 *(6)*. This paper describes the application of the method to dairy heifers.

EXPERIMENTAL PROCEDURE

The experimental animals were from the MSU dairy herd and included the Jersey, Guernsey, Holstein, Brown Swiss, and Ayrshire breeds. The average age was 4.7 months with a standard deviation of 2.3 months. They received the regular calf rations used at the University, which do not include supplemental iodine.

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[1] Approved by the Director, Michigan Agricultural Experiment Station as Journal Article No. 1935.

[2] A portion of these data is taken from a thesis by James R. Lodge to be presented to the School for Advanced Graduate Studies, Michigan State University, in partial fulfillment of the requirements for the Ph.D. degree.

A tracer dose of carrier free I^{131}, up to 150 μc, was injected subcutaneously into each heifer. The uptake of I^{131} by the thyroid gland was followed by taking counts either daily or on alternate days of the radioactivity from the thyroid area of the neck. The count used was the maximum repeatable count obtained from this region of the neck. All counts were corrected for background and the physical decay of the isotope. A Co^{60} source was used to standardize the instruments.

During most of these studies a shielded end-window TGC-8 Geiger-Mueller Tube (Tracerlab) was used with a model 1615B count rate meter (Nuclear). A scintillation counter and model 1620 count rate meter (Nuclear) were used for the later work.

Once the slope of the output curve was established, L-thyroxine, purified by repeated recrystallizations, was administered daily by subcutaneous injection. The dosages were started at a low level and increments were added each second day. An external thyroid count was taken at the end of each period on a given thyroxine level. In this way the effect of each increment upon the rate of I^{131} output by the thyroid was determined.

The thyroxine secretion rate was estimated by plotting each thyroid count, calculated as the percentage of the last previous count, against the thyroxine dosage. The amount of thyroxine required to raise the per cent of previous count to 100, or to produce a leveling off of I^{131} output to a minimum rate, was considered to represent the normal secretion rate of the thyroid gland.

RESULTS AND DISCUSSION

Thyroidal accumulation of I^{131}. The first studies were directed toward determining the rate and degree of I^{131} uptake by the thyroid gland. It might be expected that individuals will vary in both aspects, and this has been true.

Although some animals have shown a peak count on the first day after I^{131} administration, usually the highest degree of thyroid radioactivity was not reached until about 48 hours had elapsed. Many animals continued to accumulate I^{131} for a week or more after its administration.

To permit comparison of the relative per cent of the I^{131} that was taken up by the thyroid, aliquots of the injection solution were counted in a "phantom neck" similar to that described by Blincoe *(1)*.

TABLE 1

Forty-eight hour uptake of I^{131} by thyroid gland of dairy calves

Breed	Number	Average age	Uptake	Standard deviation
		(Mo.)	*(%)*	
Ayrshire	2	6.2	42.5	.....
Br. Swiss	19	4.2	33.2	18.3
Guernsey	7	6.7	47.9	21.6
Holstein	19	3.5	44.1	15.8
Jersey	16	5.8	45.8	19.9

The 48-hour uptake of I^{131} has been rather variable from one trial to another. The causes of these differences can only be speculated upon, but variations in age, breed, or season between the groups are probably major factors. The fact that the I^{131} has been administered subcutaneously rather than intravenously may also be a factor in both rate and percentage uptake.

Table 1 shows the 48-hour uptake of 63 calves. The average uptake has been 41.6% of the administered dose. The few Ayrshires and Guernseys studied and the differences in average age prevent valid breed comparisons. However, within trials, the Brown Swiss generally have been observed to have a lower 48-hour uptake than calves of similar age of the other breeds.

I^{131} output rate and biological half-life. The initial attempts to measure the thyroxine secretion rate were unsuccessful largely because of the slow rate at which the I^{131} was eliminated from the thyroids of a large proportion of the experimental animals. In some cases, there was little or no decrease in corrected thyroid counts over a 16-day period. Frequently, the counts were so erratic as to prevent the establishment of an output rate. Figure 1 illustrates these situations.

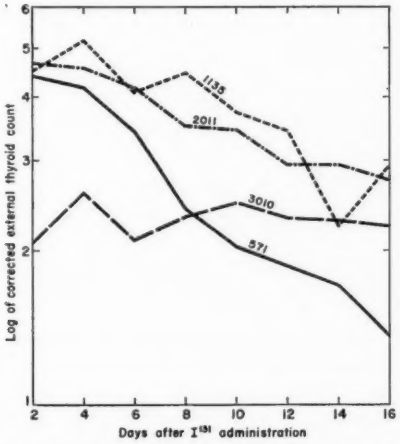

FIG. 1. The I^{131} output rate of 4 heifers. One animal appeared to have no output whereas the others showed a variable rate between counts.

A reasonable explanation for the apparent lack or irregularity of I^{131} output seemed to be that the iodine was salvaged from the metabolized hormone and recycled through the gland. The fact that these heifers had never received supplemental iodine, even though Michigan lies in a goitrogenic area, may be significant. Such animals may become particularly efficient in salvaging the small amounts of iodine contained in their feeds.

Oral dosages of 50 mg. of sodium iodide were given either daily or on alternate days to a group of calves to determine if such flooding would aid in preventing the apparent recirculation of iodine. No effect was observed when the treatment was continued for 1 to 2 weeks.

The effect of oral administration of thiouracil was then investigated because this drug prevents incorporation of iodine into thyroxine. Goldsmith *et al. (3)* have demonstrated that compounds related to thiouracil increased the excretion rate of a tracer dose of radioactive iodine from the human thyroid gland. Daily dosages of 0.2 g. of thiouracil per kilogram of body weight were used. Ely *et al. (2)* have reported that this amount administered at 12-hour intervals effectively maintains a relatively high concentration of thiouracil in the blood of calves. The thiouracil effectively prevented further incorporation of iodine into the hormone and caused a marked increase in the I^{131} output rate. It also eliminated much of the apparent variation in output rate between counts. The effect of administering thiouracil to a group of four calves starting on the 14th day after I^{131} administration is shown in Figure 2.

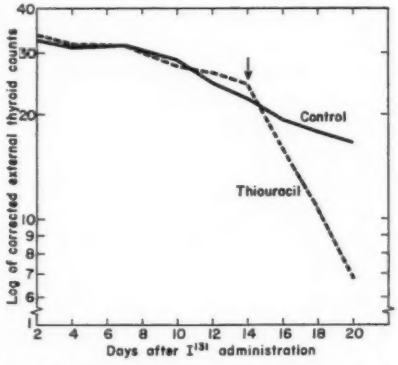

FIG. 2. The effect of administering 0.2 g. of thiouracil per kg. of body weight on the I^{131} output rate. The control and experimental groups each consisted of four calves.

The average rate at which the thyroid glands of 16 untreated and 20 thiouracil-treated calves eliminated stored I^{131} is shown in Table 2. The average rate of output was increased from 5.0 to 17.2% daily, an increase of more than threefold. This reduced the biological half-life of the isotope from 19.2 to 5.7 days.

TABLE 2

Effect of thiouracil on I^{131} output of the thyroid gland

Group	No. of animals	Per cent of I^{131} eliminated daily	Standard deviation	Days biological half-life	Standard deviation
Untreated	16	5.0	2.7	19.2	12.0
Thiouracil	20	17.2	9.2	5.7	3.8

In both thiouracil-treated and untreated groups, the output rate of Jersey calves has been more rapid than that of the other breeds. Brown Swiss calves have tended to eliminate I^{131} more slowly than the other breeds.

Thyroxine secretion rate. Two to four external thyroid counts were taken on alternate days after the start of thiouracil administration. These counts

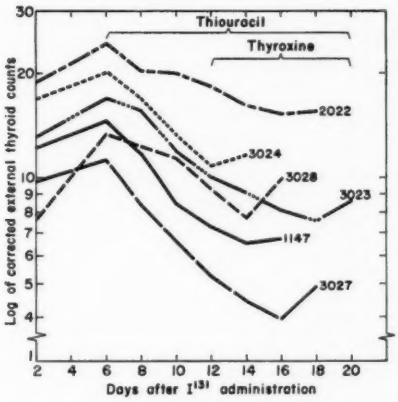

FIG. 3. Log of the external thyroid counts of 6 calves treated with thiouracil followed by thyroxine. Thiouracil was administered orally starting on the 6th day after I^{131} administration. Thyroxine in graded doses was injected subcutaneously daily starting on the 12th day with increments added each second day.

were used to establish the slope of the output curve. Typical results from a trial involving six heifers are shown graphically in Figure 3. Immediately after the last count, a low dosage of thyroxine was administered subcutaneously. On the basis of preliminary studies, this initial level had been set at 0.3 mg. per 100 lb. of body weight. In most cases little or no effect upon the output of I^{131} has been observed from this level. The dosage was repeated the next day, and a count was taken on the following day, at which time the dosage was increased. Thus the animals received two daily injections at each level of thyroxine, and thyroid counts were taken at the end of the period. In the trials reported here increments of 0.1 mg. of thyroxine were used. However, with the more sensitive instruments now in use, it may be possible to lengthen the counting period and to decrease the increments to 0.05 mg. or less per 100 lb. of body weight.

As the thyroxine dosage was progressively increased, its inhibitory effect upon the thyroid gland was marked by a decreased rate of I^{131} output by the thyroid. The thyroxine secretion rate was estimated by plotting each thyroid count, expressed as the per cent of previous count, against thyroxine dosage (Figure 4). The amount of thyroxine required to raise this figure to 100% has been taken as representing the animals' thyroxine secretion rate.

The results of two trials, one in May and the other in late August and early September, are shown in Table 3. The secretion rates have ranged from 0.47 to 0.84 mg. per 100 lb. body weight, and the values obtained in the August-September trial were generally lower than those in May. These findings fit the usual assumption that thyroid activity is depressed during hot weather.

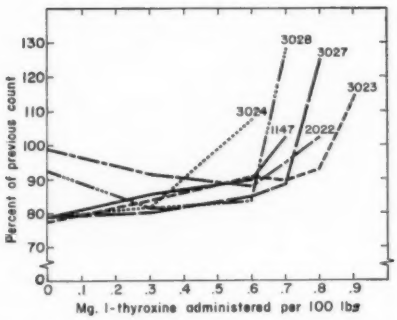

FIG. 4. Corrected external thyroid counts calculated as the per cent of the last previous count. The daily amount of thyroxine required to raise the per cent of previous count to 100 was assumed to represent the daily secretion rate of the thyroid gland.

TABLE 3

Estimated thyroxine secretion rate

Calf No.	Breed	Age	Month	Thyroxine secretion rate	
		(Mo.)		*(Mg/cwt.)*	*(Mg/calf)*
1147	J	7.5	May	.78	2.46
2022	G	7.0	May	.77	2.73
3023	BS	6.5	May	.84	3.32
3024	BS	5.5	May	.51	2.09
3027	BS	4.0	May	.73	2.30
3028	BS	4.0	May	.64	1.86
596	H	5.0	Aug.-Sept.	.57	1.91
598	H	4.5	Aug.-Sept.	.53	1.56
1151	J	5.0	Aug.-Sept.	.47	1.15
1153	J	4.5	Aug.-Sept.	.57	1.48
3031	BS	5.5	Aug.-Sept.	.60	2.43
3032	BS	5.0	Aug.-Sept.	.54	1.89
3033	BS	3.5	Aug.-Sept.	.56	1.40

SUMMARY

A thyroxine substitution technique for measuring the daily thyroid secretion rate of intact dairy calves is described. The 48-hour uptake of tracer amounts of I^{131} injected subcutaneously averaged 41.6% of the administered dose for 63 calves. Although the uptake varied between individuals and trials, Brown Swiss heifers had a comparatively lower uptake than the other breeds.

The experimental animals, none of which received supplemental iodine in their ration, demonstrated a considerable capacity to recycle iodine through the thyroid. This could not be prevented by subcutaneous administration of potassium iodide during the experimental period but was avoided when thiouracil at the rate of 0.2 g. per kilogram of body weight was given orally.

The daily thyroxine secretion rate of 13 calves ranged from 0.47 to 0.84 mg. per 100 lb. of body weight daily. Values obtained in August-September averaged lower than those in May.

ACKNOWLEDGMENT

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A STUDY OF THE SECRETION OF IODINE IN MILK OF DAIRY COWS, USING DAILY ORAL DOSES OF I^{131} [1,2]

F. W. LENGEMANN [3] AND E. W. SWANSON

University of Tennessee — Atomic Energy Commission, Agricultural Research Program Oak Ridge, Tennessee, and
Dairy Department, University of Tennessee, Knoxville

The authors have traced the secretion of iodine to the fat and nonfat fractions of milk, through the normal pathways of excretion and to its retention in the blood and thyroid. They have also demonstrated the usefulness of the isotope dilution method for studying trace mineral metabolism. *Editor.*

The metabolism of iodine by the dairy cow has been of interest for many years. However, owing to the lack of reliable techniques adaptable to routine analyses, the secretion of iodine into milk has not been studied in detail. The advent of nuclear fission markedly increased the amount of research concerning iodine metabolism by providing radioactive nuclides which, though chemically indistinguishable from the nonradioactive iodine, are readily detected by their reactions. Also, the observation of Van Middlesworth *(16)* that radioactive iodine with a half-life of 8 days could be detected in the thyroids of cattle in many parts of the country in the summer of 1954, made it of more than academic interest to learn more about the secretion of iodine into milk.

The secretion of I^{131} in milk has been studied by Honour *et al. (7)* in humans, in a rabbit and goats by Wright *et al. (19)*, and in a dairy cow by Glascock *(3)*. All of these studies charted the fate of a single administration of I^{131}. Since exposure of grazing animals to radioactive fall-out is likely to be for a period of several days, it is desirable to know how continuous administration affects the secretion of I^{131} in milk.

The daily administration of a radioactive isotope has been used by Van Middlesworth *(17)* to study iodine metabolism in the rat, by Visek *et al. (18)* using Ca^{45} in dairy cattle, and by Comar *et al. (2)* with Sr^{90} in rats.

The experiments reported in this paper show a comparison of the results of single administrations of I^{131} with the results of daily dosing experiments. An

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[2] This work was completed under Contract No. AT-40-1-GEN-242 between the University of Tennessee, College of Agriculture, and the Atomic Energy Commission. The radioactive materials used in this work were obtained from the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission.

[3] Present address: Division of Chemistry, University of Tennessee, Memphis.

attempt also was made to reduce the radioactive iodine content of milk by giving massive oral doses of sodium iodide. The data of the continuous dosing experiments are used to demonstrate the possibilities of using this technique in studying iodine metabolism.

EXPERIMENTAL METHODS

Two grade Jersey cows were used for this study. Cow A was 8 years old, about 3 months pregnant, and was producing 6.6 l. of milk per day. Cow B was 6 years old, about 4 months pregnant, and was producing 7 l. of milk per day. Both cows had been lactating for nearly a year. Each cow was fed daily 12 lb. of commercial dairy ration (18% protein) and mixed hay. Milkings and feedings took place at 8 A. M. and 4:30 P.M. every day. Throughout the collection periods the cows were confined in metabolism stalls similar to those described by Hansard *et al. (6)* and Comar *(1)*. These were altered by using a swinging stanchion instead of a fixed one, using a slip-proof coating on the floor, and providing a removable panel in the side to facilitate milking the cows. During the daily dosing experiments each cow was dosed at 4 P.M. for periods of at least 21 days. Doses were given in gelatin capsules by means of a balling gun. To prepare the dosing capsule, 1 ml. of a stock solution containing 1 mc. of carrier-free I^{131} (as NaI) was dried onto tissue paper contained in the gelatin capsule. All the capsules for a given experiment were prepared on the same day.

Samples of milk were taken at every milking. Blood was collected, and the radioactivity of the thyroid was determined every day at 10 A.M. When the blood and milk concentrations of I^{131} remained constant for 4 days, it was assumed that the major portion of the body iodine was in equilibrium with the dietary iodine. Daily urine and feces samplings were taken at this time. The cows were then fed large amounts of stable iodide to try to change the amount of I^{131} appearing in the milk. Cow A received a capsule containing 2 g. of iodine (as NaI) and cow B received 4 g. of iodine in addition to the daily dose of I^{131}. For comparison with the continuous dose trials, a 7-day balance trial was made with each cow after she had received only a single 5-mc. oral dose of I^{131}. The conditions were the same as before except that samples of urine and feces were taken every 24 hours.

To determine the concentration of I^{131} in the samples, an accurately measured quantity, from 0.5 to 3.0 ml., of the liquids was pipetted into a 16 × 150 mm. test tube. For solid material, from 1 to 2 g. was weighed into a test tube. Radioactivity was counted with a scintillation well counter containing a thallium-activated sodium iodide crystal. All results were corrected for decay by use of activity standards and expressed as per cent of the daily dose or, in the single dose experiments, as per cent of the dose.

For thyroid measurements a sensitive scintillation probe was used. It contained a thallium-activated sodium iodide crystal and was fitted with a brass sleeve that kept the crystal a constant 8 inches from the thyroid during measurements. The probe was connected to a count-rate meter, and the highest steady

reading was determined. These readings were corrected for body background and standard counts.

In some instances blood and milk samples were fractionated. The whole blood was divided into plasma and blood cells by centrifugation. The plasma proteins were precipitated with 20% trichloracetic acid. Any I^{131} that was found with this protein fraction after a second washing with 20% trichloroacetic acid was considered to be protein-bound and that in the supernatant was considered to be inorganic iodide. Milk was centrifuged to separate cream and skimmilk. The cream was made alkaline with NH_4OH and extracted with ethyl ether and petroleum ether. This was considered to be the ether-soluble fraction. That portion of the cream not soluble in ether was considered to be the nonfat fraction. The skimmilk was treated with 20% trichloroacetic acid to precipitate the protein-bound iodine, and the supernatant was considered to contain only inorganic iodide.

TABLE 1

Distribution of a single 5 mc. oral dose of I^{131} in the blood, milk, urine, and feces, and the ratios of urine I^{131} to feces I^{131} from cows A and B

Cow	Total per cent of dose in 7 days			Ratio of feces I^{131} to urine I^{131}	
	Milk	Feces	Urine	First day	Seventh day
A	6.62	13.4	73.1	0.08	0.31
B	5.96	23.1	73.5	0.25	0.45

RESULTS

The distribution of a single oral dose of I^{131} from cows A and B is presented in Table 1. About 6% of the radioisotope was found in the milk, and 73% was excreted in the urine during the 7-day period. From the disappearance curves of the milk I^{131}, it was calculated that the concentration decreased 50% in 1.25 days for cow A and in 1.35 days for cow B. The ratio of feces I^{131} to urine I^{131} was used to express the changing pattern of I^{131} excretion noted during such trials. These ratios indicate that as the time after dosing increased a larger percentage of the excreted I^{131} was being channeled into the feces.

During these experiments and the continuous dosing experiments a feed balance and an iodine analysis, according to the method of Raben (*13*), of the ration showed that each cow received 15 mg. of iodine daily. The activity in the blood and milk of these cows during the first phase of the daily dosing trials is shown in Figure 1. It is to be noted that the blood and milk values reached a plateau at the sixth day and then remained constant for 4 days. This indicated that most of the metabolically active body iodine had equilibrated with the dietary iodine. The thyroid accumulation of I^{131} of both animals during this period is shown in Figure 2. At 6 days the thyroids had not reached a peak in the acquisition of radioiodine but the increase during the next 4 days was very small. At the tenth day the thyroid of cow A accumulated only about 1% and the thyroid of cow B less than 3% of the I^{131} contained in the ninth capsule.

The data collected during daily oral doses are presented in Table 2. The data are expressed as per cent of a daily dose. The high thyroid values reflect the

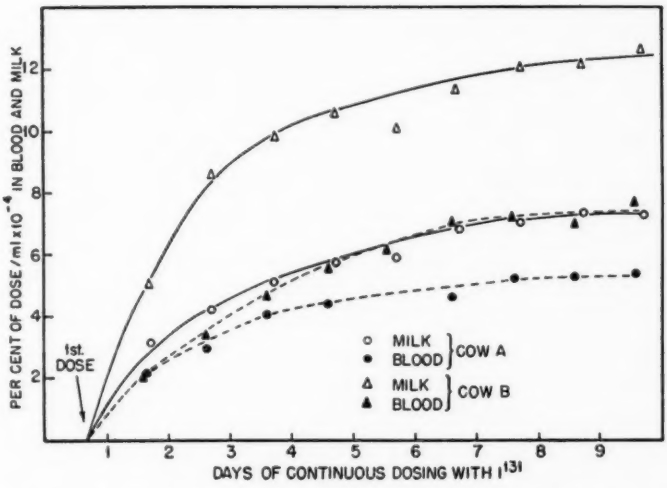

FIG. 1. Concentration of I^{131} in milk and blood of cows A and B after the start of daily doses of I^{131}.

ability of this gland to store iodine. Cow A secreted about 4.7% of a daily dose into the milk each day. This is somewhat lower than the 6.6% collected during the 7-day single dose trial. For cow B an opposite result was noted, in that 9.4% was in the milk each day during the continuous administration but only 6.0% was collected in 7 days after a single dose. The increase in importance of the digestive tract as an excretory pathway for iodine is of interest.

At the tenth day the administration of daily massive doses of NaI in addition to the regular I^{131}-containing capsule began. Within 2 days cow A, receiving 2 g. of iodine per day, showed a decrease of 27% in the concentration of I^{131}

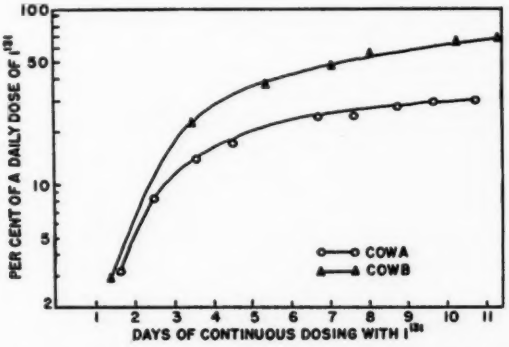

FIG. 2. Accumulation of I^{131} in the thyroids of cows A and B after the start of daily doses of I^{131}.

TABLE 2

The distribution of I^{131} in blood, milk, urine, feces, and thyroids of cows A and B after equilibrium was reached during daily oral dosing with I^{131} and after the effect of combining massive doses of NaI with the daily I^{131} dose

Cow	Iodine intake daily	Per cent of daily dose				Per cent per liter	
		Milk	Feces	Urine	Thyroid[a]	Milk	Blood
A	15 mg.	4.7	37.8	34.6	30	0.74	0.54
A	2 g.	3.3	—	—	9[b]	0.54	0.74
B	15 mg.	9.4	22.0	50.3	65	1.26	0.75
B	4 g.	4.5	16.8	102.6	22[c]	0.62	1.17

[a] This expresses the accumulated I^{131} on the basis of a daily dose.
[b] Level reached 9 days after the start of administration of 2 g. of I.
[c] Level reached 10 days after the start of administration of 4 g. I.

in the milk while the blood level increased 37% (Table 2). Cow B, given 4 g. of iodine per day, showed a decrease of 51% in the activity level of the milk while that of the blood rose 56%. The new low levels of I^{131} in the milk were maintained for at least 5 more days. The high doses of stable iodide seemed to have a flushing effect on the I^{131} accumulated in the body. Table 2 shows that cow B, at 2 days after the first massive dose of NaI, excreted an amount of I^{131} equivalent to 120% of a daily dose, and nearly all of the extra I^{131} was found in the urine. The massive iodide supplement also served to remove I^{131} from the thyroid. In cow A the thyroid level of the isotope decreased 77% in 9 days and in cow B 66% in 10 days. These levels probably would have decreased still more had the high iodide feeding continued.

Tables 3 and 4 show the distribution of I^{131} in the blood and milk during the daily dosing trials. The major effect of iodide supplementation was to decrease the proportion of I^{131} associated with the protein and blood cells and increase the inorganic I^{131} of the blood. Little change was observed in milk, with most of the activity being found in the inorganic fraction. Cheese and fresh cream butter were made from the milk produced by cow A 10 days after the start of the daily I^{131} administration (normal iodide level). It was found that 0.01% of a daily oral dose was in the butter and 0.26% in the buttermilk. About 0.9% of the I^{131} in the milk (5% butterfat) was incorporated into the butter and could

TABLE 3

Distribution of I^{131} in the fractions of blood and of milk after 10 days of continuous daily doses of I^{131} on normal feed, and again after giving daily massive doses of NaI

Cow	Iodine intake daily	Per cent of whole blood I^{131}			Per cent of milk I^{131}			
			Plasma		Cream		Skimmilk	
		Blood cells	Protein-bound	Inorganic	Ether sol.	Non-fat	Protein-bound	Inorganic
A	15 mg.	3.8	14.7	83.8	0.05	2.7	4.7	91.4
A[a]	2 g.	7.4	1.6	91.4	0.0	3.9	3.5	92.7
B	15 mg.	4.5	22.0	71.4	0.0	4.4	13.1	81.7
B[b]	4 g.	3.9	11.8	84.1	0.0	3.3	8.3	88.5

[a] Sample collected 9 days after the start of the massive NaI doses.
[b] Sample collected 5 days after the start of the massive NaI doses.

TABLE 4

The concentrations of iodine in milk, whole blood, blood plasma, and thyroids of cows A and B, as calculated from the I^{131} content after attainment of equilibrium

Cow	Iodine intake daily	Milk	Whole blood	Plasma (Protein-bound)	Thyroid
		——— *γ Iodine per 100 ml.* ———			*(mg.)*
A	15 mg.	11.1	8.1	1.2	4.5
A	2 g.	1910	1480	23.7	—
B	15 mg.	18.9	11.3	2.5	9.7
B	4 g.	2480	4680	552	—

not be washed out. Data on cheese, a rennin coagulate, indicated that 12.7% of the I^{131} of milk would be found in a whole milk cheese containing 40% moisture.

In an effort to repeat the daily dosing experiment with cow A, an effect which may have been caused by seasonal variation was encountered. The first experiment was made in February. In the second experiment, started after the middle of April, an equilibrium level of I^{131} in the blood and milk was not reached until 11 days. This was 1.6 times longer than in the first experiment. The milk I^{131} level was 2.9 times greater than in the first trial.

DISCUSSION

The results from the daily dosing experiment agreed well with the calculations of Glascock *(3)* from single oral dose data that a constant level of I^{131} in the milk would be reached in one week under conditions of daily ingestion. Probably because of the differing techniques, his prediction that the milk level of the isotope would be 2.3 times the maximum observed in a single dose trial was not confirmed. The observation that cow A secreted much less and cow B much more I^{131} in a 24-hour period during the continuous dosing experiment than was found in the milk during a 7-day collection after the single oral dose, makes it difficult to convert the data of single administration experiments to conditions of prolonged exposure. This also indicated the need for procedures that simulate field conditions.

These results show that less than 10% of ingested I^{131} would normally get into the milk of the dairy cow. Of this, only about 1% of the activity of the milk would be found in butter and less than 15% in the cheese manufactured from this milk. The short half-life of iodine^{131} (8 days), the delay, and the dilution that any particular sample of milk encounters on its way to the consumer would reduce the concentration of activity even more. The fact that humans are known to excrete 60% of ingested I^{131} within 2 days (*8*) tends to remove I^{131} from milk from consideration in estimating potential hazards in the event of contamination of the feed supply by small amounts of this isotope.

The use of massive doses of sodium iodide to cattle exposed to I^{131} would not be practical if the purpose were to reduce the I^{131} content of the milk. As the data show, increasing the dietary iodide more than 200 times reduced the I^{131}

content of the milk by only one-half. If, however, the purpose were to remove the I^{131} from the animal's thyroid and other body tissues, then the supplementation would be of value. Data from cow B showed that stable iodide, probably acting through exchange, had a flushing effect upon the I^{131} accumulated in the body and thyroid. This effect would reduce the length of time required for the I^{131} levels to fall to very low values. Increased ingestion of stable iodide has been suggested by Hamilton (*1*) as a method of protecting against small intakes of radioactive iodine.

Continuous ingestion of I^{131} has been demonstrated as a technique to study the normal metabolism of iodine. One advantage is that less activity can be used in each dose than for single dose experiments. If, as was done here, a single dose of 5 mc. is given orally, then it would not be unusual to find from 1.5 to 2 mc. being eliminated from the body within 24 hours. In daily dosing trials, where the initial dose contained 1 mc., the maximum amount contained in all body secretions and excretions at equilibrium would be about 0.5 mc. This lower level would present less of a problem in handling experimental animals.

Continuous dosing with I^{131} more accurately depicts conditions under which iodine would normally be metabolized than does a single dose of radioiodine. In a single dose trial most of the activity is excreted in the urine. However, a trend is seen as the time after dose continues of an increasing proportion being excreted in the feces. In the trials reported here, using the continuous dosing method, the kidney was not always the primary organ of excretion. Since the bile has been shown to be a pathway of excretion of bound iodine (*15*), the excretory patterns during continuous dosing may be due to the more complete distribution of I^{131} during the long-term trial.

An important possibility of the daily dose technique is its substitution for difficult chemical analysis. This is particularly true for iodine which has a very small storage or circulation in the body. If the intake of the radioisotope and of the stable element are maintained in a constant proportion, the ratio of the radioactive to stable isotopes after a period of time will be the same in all parts of the body and in all secretions and excretions. Measurement of the radioelement will then be an accurate indicator of the metabolism of the natural element. Since the proportion (specific activity) becomes a constant, one estimate of this ratio, either in the diet or in a tissue or fluid, can be used as that prevailing in any part of the body. Combining the measurement of the radioactivity of a sample with the specific activity allows the concentration of the stable element to be calculated. Since the activity measurements in these trials were always expressed as fraction of the daily dose, this formula was used: Total iodine concentration of X = (Total mg. of I supplied daily) (Fraction of a daily dose of I^{131} in X), in which X represents the sample measured, i.e., blood, milk, urine, saliva, etc.

Calculations of the total iodine content of some tissues and products were made using the data of Tables 2 and 3 and are presented in Table 4. The fact that blood and milk levels were constant for four days and that the thyroids were

accumulating extremely little I^{131} was used as evidence that the iodine pools of the body were uniformly labeled. Van Middlesworth (*17*) in a similar procedure with rats considered all functional iodine pools to be uniformly labeled when the daily urine and feces excretion equaled the daily dose. At this time it was also observed that the feed and thyroid specific activities were equal. The recovery of the daily dose from excretions, as shown in Table 2, from continuously dosed cows was only 77 and 82%. The thyroid was still increasing slightly in I^{131}, and other avenues of loss were possible besides those collected, e.g., hair, saliva, tears. Therefore, it has been assumed that all of the iodine pools of significant size (especially those of the blood, mammary gland, and thyroid) were near equilibrium at the time the samples for calculations were taken.

The calculated values for the iodine content of milk while the cows were receiving daily 15 mg. of iodine fall within the range found by Hanford and Supplee (*5*) for cows on iodine intakes up to 50 mg. per day and are slightly higher than those of Remington and Supplee (*14*) for 117 samples of milk from cows consuming about 3 to 5 mg. of iodine per day. The values calculated for whole blood (Table 4) are higher than similar values reported by Matthews *et al. (12)* for normal cows but lower than their values from iodine-supplemented cows. The plasma protein-bound iodine values calculated here (Table 4) for cows are lower than the averages given by Lewis and Ralston *(9)* and Long *et al. (10)* for different groups of Jersey cows, viz., 4.9, 3.3, and 4.1 γ per 100 ml. of plasma, but are in the same range as the values observed for beef breeds. In view of the advanced ages and stages of lactation of the animals used here, the low calculated values may be expected. The values observed after excessive doses of iodide (Table 4) are comparable to those reported by Long *et al. (11)* after feeding iodinated casein containing excess iodine.

From these calculations it appears that the supplementary iodine did not have a greater effect on the percentage of I^{131} appearing in milk because the excess iodine was quite readily absorbed into the body and passed into the milk in addition to a nearly normal complement of I^{131}. However, the data of Table 2 indicate that at the extremely high dietary iodine levels there appeared to be some mechanism that operated to keep the milk iodine level relatively lower than that of the blood. Another feature of the continuous dosing technique is that it provides a method for estimating accurately the iodine content of the thyroid in the intact live animal. This determination would have many applications in studies of iodine metabolism and thyroid function.

SUMMARY

Daily oral doses of I^{131} to dairy cows have been used to simulate conditions of prolonged exposure to radioactivity. Maximum levels of I^{131} were found at the seventh day in milk and blood and after the tenth day for the thyroids. No simple formula could be applied to extrapolate data from single-dose trials to conditions of continuous ingestion of the radionuclide.

From 5 to 10% of a daily dose appeared in the milk each day during continuous I^{131} ingestion. Massive daily doses of sodium iodide reduced the radioactivity of the milk by one-half in two days and removed two-thirds of the isotope from the thyroid in 10 days.

Calculations of the total stable iodine content of milk, blood, and thyroid were made to demonstrate the usefulness of this variation of the isotope dilution method for studying trace mineral metabolism.

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EXTRA-PERIOD LATIN-SQUARE CHANGE-OVER DESIGNS[1]

H. L. LUCAS

Department of Experimental Statistics, North Carolina State College, Raleigh

An extra period of observation has been added to the Latin-square change-over designs for large-animal experiments, in order to estimate carry-over effects more effectively. Treatments in the extra period are the same as in the last period of the Latin-square. *Editor.*

Change-over designs of the Latin-square or round-robin type have been used for many years in research with milking dairy cattle. The basic pattern of these designs may be illustrated by the following setup for three treatments.

Experimental period	Treatment sequence		
	1	2	3
1	1[a]	2	3
2	2	3	1
3	3	1	2

[a] The numbers in the heart of the table represent the treatments.

One or more animals, ordinarily an equal number, are placed on each treatment sequence. Various alternative Latin-square arrangements may be used, and the patterns may be extended to more than three treatments. These change-over trials have found much favor in dairy-cattle work. They permit comparison of treatments on a within-cow basis, thus eliminating from experimental error the between-cow differences in production level.

One of the complications of change-over trials is that performance on a given treatment may be affected by the treatments administered earlier in the same sequence. A method often used to eliminate these carry-over or residual effects was to base interpretations on the performances during only the latter portions of the experimental periods. In 1941, however, Cochran *et al.* (*1*) described certain Latin-square patterns and a method of analysis which permit the estimation of residual effects without deleting the performances from the earlier portions of the periods. These patterns require that each treatment be preceded by every other treatment an equal number of times, and are said to be "balanced" for

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residual effects. For example, with three treatments, the following two squares (six sequences) are required to attain balance:

1	2	3	1	2	3
2	3	1	3	1	2
3	1	2	2	3	1

In general, balance can be attained with a minimum of two squares when the number of treatments is odd and with one square when the number of treatments is even. Other numbers of squares may also be used as long as the balance is maintained.

The designs just discussed are constructed and analyzed on the assumption that the residual effects of a treatment carry over only one period. Williams (*6, 7*) has studied designs which permit estimation of residual effects that last more than one period, devoting particular attention to carry-over into two periods. Patterson (*3, 4*) has published series of incomplete Latin-squares (fewer periods than treatments) that are balanced for one-period carry-over effects.

In all of the published series of designs, the precision with which residual effects are estimated is considerably less than that with which the direct effects of treatments are estimated. This is in part because the residual effects are replicated fewer times than are the direct effects, but also in large share because the residual effects are nonorthogonal both to sequences and to direct effects. The designs to be presented in this paper overcome almost entirely this undesirable aspect of the Latin-squares, when they are analyzed under the assumption that carry-over persists only one period.

THE DESIGNS

In a seminar given at North Carolina State College in 1947, F. Yates (Rothamstead, England) pointed out that the precision of estimating one-period residual effects in the Latin-square designs could be considerably improved if all sequences were followed by a check treatment. B. G. Greenberg (Department of Biostatistics, University of North Carolina, Chapel Hill) then mentioned to the author that the extra period would be used more efficiently if some pattern of the experimental treatments were employed instead of a check treatment. Subsequent study showed that the optimum arrangement was provided simply by repeating the last row of the Latin-square designs.[a] Thus, the designs are easily formed from any of the complete Latin-squares balanced for one-period residual effects. Useful patterns are given below. The rows represent experimental periods and the columns represent the treatment sequences.

TREATMENT PATTERNS

Two treatments:

This pattern must be repeated at least once in order to obtain an estimate of error.

1	2
2	1
2	1

Three treatments:

The orthogonal set of squares. (Use complete set.)

1	2	3		1	2	3
2	3	1		3	1	2
3	1	2		2	3	1
3	1	2		2	3	1

Four treatments:

The orthogonal set of squares. (Use complete set.)

1	2	3	4		1	2	3	4		1	2	3	4
2	1	4	3		3	4	1	2		4	3	2	1
3	4	1	2		4	3	2	1		2	1	4	3
4	3	2	1		2	1	4	3		3	4	1	2
4	3	2	1		2	1	4	3		3	4	1	2

The balanced single square:

1	2	3	4
2	4	1	3
3	1	4	2
4	3	2	1
4	3	2	1

Five treatments:

The orthogonal set of squares. (Use complete set.)

1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
2	3	4	5	1		3	4	5	1	2		4	5	1	2	3		5	1	2	3	4
3	4	5	1	2		5	1	2	3	4		2	3	4	5	1		4	5	1	2	3
4	5	1	2	3		2	3	4	5	1		5	1	2	3	4		3	4	5	1	2
5	1	2	3	4		4	5	1	2	3		3	4	5	1	2		2	3	4	5	1
5	1	2	3	4		4	5	1	2	3		3	4	5	1	2		2	3	4	5	1

Balanced pairs of squares. (Use one or both pairs.)

Pair 1

1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
2	5	4	1	3		5	3	1	2	4		2	4	1	5	3		3	1	5	2	4
3	4	2	5	1		2	5	4	1	3		3	1	5	2	4		2	4	1	5	3
4	1	5	3	2		4	1	5	3	2		4	5	2	3	1		5	3	4	1	2
5	3	1	2	4		3	4	2	5	1		5	3	4	1	2		4	5	2	3	1
5	3	1	2	4		3	4	2	5	1		5	3	4	1	2		4	5	2	3	1

[a] Institute of Statistics Mimeograph Series No. *18*, University of North Carolina, 1948.

There are several interesting points of contrast between the extra-period and the regular Latin-square designs. In the extra-period designs each treatment is preceded by every other treatment as in the Latin-squares, but, in addition, each treatment is preceded by itself. This renders the one-period residual effects orthogonal to the direct effects. In contrast to the Latin-squares, the residual effects in the extra-period designs are orthogonal to sequences. The direct effects are nonorthogonal to animals in the extra-period patterns, but the degree of nonorthogonality is not great. Finally, the amount of replication of the residual effects relative to that of the direct effects is a little greater than in the Latin-squares. The reduction in nonorthogonality results in a somewhat simpler analysis for the extra-period designs than for the Latin-squares.

Presumably, the extra-period idea might be applied to improve any of the designs balanced for carry-over effects, whether the designs be balanced for carry over into one or into more periods, or whether the squares be complete or incomplete. More than one extra period might be called for when residual effects are assumed to continue for more than one period. Such possibilities were not developed, however, in these studies.

USE OF THE DESIGNS

General considerations. Patterns may be written easily for six or more treatments, but the number of experimental periods becomes too great to be of much use in work with dairy cattle. Ordinarily, one will wish to use cows between peak lactation and mid-gestation. Thus, if each experimental period is to be several weeks in length, the number of periods that can be employed will be limited. If desired, designs for six or more treatments may be obtained by referring to Williams (*6, 7*) and Patterson (*3, 4*). The advantage of the extra-period designs over the regular Latin-square designs decreases as the number of treatments is increased. In fact, when the number of treatments is six or more, the incomplete Latin-squares (*4*) likely would be chosen, simply to avoid using too many periods. Also, the extra-period designs are less efficient (i.e., provide less information per animal per period) than the Latin-squares, if residual effects can be assumed not to exist. For this reason, the Latin-squares might be preferred to the extra-period designs, even when studying few treatments, if it can be assumed safely that residual effects are negligible. If residual effects are negligible, however, it may be preferable, because of their low errors, to use the switch-back designs for two or more treatments (*2, 5*), instead of either the Latin-square or the extra-period designs.

Repetitions and combinations of patterns. It is convenient for the present discussion to consider that a complete basic design or pattern consists of one of the complete patterns previously given, with but one animal allotted to each sequence of the pattern. It is taken in the method of analysis that a square consists of a single square from a pattern, with only one animal allotted to each sequence in the square. The method of analysis permits the number of squares used in an experiment to vary, but requires that the squares used represent repetitions of complete basic designs or combinations of complete basic designs.

The number of squares used in an experiment will be dictated to a considerable extent by the sensitivity to be attained. Sensitivity in turn is determined in part by the size of basic experimental error, in part by the number of observations per treatment and by the degrees of freedom for error. Ordinarily, at least five or six repetitions of the two-treatment design, and at least two repetitions of the three-treatment or of the four-treatment single-square design, will be desired. As an example of combining different basic designs, consider that the desired sensitivity calls for four squares in comparing four treatments. In this instance, one could use the orthogonal set plus the balanced single square. In general, it will be noted that, regardless of the number of repetitions or of the particular combinations of basic patterns used, the total number of squares involved must

be even when the number of treatments is odd, but can be either even or odd when the number of treatments is even.

Choice of squares and allotment of treatments and animals. Little need be said about choice of squares. For two and three treatments the patterns given are the only balanced ones that can be formed. For four and five treatments one simply needs to choose at random between the few possible ways of repeating and/or combining complete patterns that provide the desired total number of squares. Having so chosen, no rearrangement of rows to periods should be made, as this will upset the balance of the designs. The assignment of treatments to numbers is made at random, one assignment serving for all squares to be used in the experiment. The animals should be allotted to the sequences of treatments at random, but ordinarily it will be advantageous to partially restrict the randomness of this allotment, as is explained in the following paragraphs.

The experimental error associated with the method of analysis to be given, stems in large share from the variations of the general time trends between cows within squares. The decline in production of high-producing cows is usually greater in absolute amount than is the decline of low producers. This being the case, it is efficacious to insure that the animals assigned to a given square are as uniform as possible with respect to initial production level. The groups of different production levels are then assigned randomly to the different squares, with subsequent random allocation of the cows in a group to the sequences of the assigned square. This point has been made by Cochran *et al.* (*1*).

As an example of the assignment of cows to squares and sequences, assume that the orthogonal set of squares for four treatments is to be used. The twelve cows would be divided into high, medium, and low producers. The three groups would then be assigned at random to the three squares, with subsequent allotment of animals within groups to sequences within squares. As another example, assume that the pattern for three treatments is to be used in duplicate, thus requiring twelve cows. The cows would be divided into four groups according to production level. The two higher-producing groups would first be assigned at random to the two squares of the pattern; then, on an independent random draw, the two lower-producing groups would be assigned to the two squares. After assignment of the groups to squares, the animals within groups would be assigned to sequences within squares.

The method of allotment of cows to squares and sequences just described strictly applies only when all cows can be started on experiment at the same time. This is an ideal situation, which tends to insure a minimum experimental error because it permits maximum homogeneity within groups with regard to production level. Sometimes, however, the freshening and pregnancy statuses of the animals are such that all squares cannot be started at the same time. In such an event, the squares would be assigned to starting groups at random, and the cows in each starting group would be assigned at random to the sequences of the corresponding square.

Blocking of designs. The method of analysis permits the subdivision of the total number of squares into blocks. The number of squares need not be the

same in all blocks, but a block must consist of a complete pattern, or of repetitions or combinations of complete patterns. For two treatments a block should consist of at least two squares.

The blocking feature might be useful, if, by desire or by necessity, the treatments are to be compared under more than one set of basal conditions. For example, in order to examine the possibility of a treatment by breed interaction, it might be desired to use one breed of cows to form one block of squares and another breed to form a second block. In another instance, sufficient total replication might be attainable only if one block is placed at one farm and other blocks at other farms.

STATISTICAL ANALYSIS

Although several aspects of the analysis of the extra-period designs follow standard procedures, all computations will be outlined for the convenience of the user.

Definition of symbols and preliminary computations. Let

Y_{ijk} = the performance of the j^{th} animal in the i^{th} square during the k^{th} period ($i=1, 2, \ldots, q$; $j=1, 2, \ldots, n$; $k=1, 2, \ldots, n+1$)

M = the grand sum of the Y's

G_i = the sum of the Y's in the i^{th} square

P_{ik} = the sum of the Y's in the k^{th} period of the i^{th} square

Z = the grand sum of the Y's less the sum of the Y's occurring in the first period

C_{ij} = the sum of the Y's for the j^{th} animal in the i^{th} square

D_h = the sum of the Y's occurring on the h^{th} treatment ($h=1, 2, \ldots, n$)

R_h = the sum of the Y's immediately following the h^{th} treatment (note that this includes observations in the last period, even though treatment in the last two periods is always the same)

L_h = the sum of the C's for all animals that received the h^{th} treatment in the last two periods

$W_h = (n+1)D_h - L_h - M$ (Note: $\sum_h W_h = 0$)

If the design is blocked, additional quantities are needed, as follows:

q_u = the number of squares in the u^{th} block ($u=1, 2, \ldots, m$)

M_u = the sum of all Y's in the u^{th} block

Z_u = the sum for the u^{th} block of all Y's except those occurring in the first period

D_{uh} = the sum of the Y's occurring on the h^{th} treatment in the u^{th} block

R_{uh} = the sum of the Y's immediately following the h^{th} treatment in the u^{th} block

L_{uh} = the sum of the C's for all animals in the u^{th} block that received the h^{th} treatment in the last two periods

$W_{uh} = (n+1)D_{uh} - L_{uh} - M_u$ (Note: $\sum_h W_{uh} = 0$ for each block)

Analysis of variance. The key-out and the computational formulas to obtain the various sums of squares required in the analysis of variance are shown in Table 1. Mean squares are computed and tests of significance made in the usual manner.

TABLE 1

Key-out and computational formulas for the analysis of variance

Situation	Variance source	Degrees of freedom	Abbreviation	Computational formula
Either blocked or not blocked	Correction factor	1	CF	$M^2/qn(n+1)$
	Total	$qn(n+1)-1$	TSS	$\sum_i \sum_j \sum_k Y^2_{ijk} - \text{CF}$
	Squares	$q-1$	SSS	$\frac{1}{n(n+1)} \sum_i G^2_i - \text{CF}$
	Periods within squares	qn	SSP	$\frac{1}{n} \sum_i \sum_k P^2_{ik} - \text{SSS} - \text{CF}$
	Animals within squares	$q(n-1)$	SSC	$\frac{1}{n+1} \sum_i \sum_j C^2_{ij} - \text{SSS} - \text{CF}$
Not blocked	Direct effects of treatment	$n-1$	SSD	$\frac{1}{qn(n+1)(n+2)} \sum_h W^2_h$
	Residual effects of treatment	$n-1$	SSR	$\frac{1}{qn} \sum_h R^2_h - \frac{1}{qn^2} Z^2$
	Error	$(qn-2)(n-1)$	SSE	TSS − SSS − SSP − SSC − SSD − SSR
Blocked	Direct effects	as for the not-blocked situation		
	Residual effects			
	Direct effects by blocks	$(m-1)(n-1)$	SSDB	$\frac{1}{n(n+1)(n+2)} \sum_u \frac{1}{q_u} \sum_h W^2_{uh} - \text{SSD}$
	Residual effects by blocks	$(m-1)(n-1)$	SSRB	$\frac{1}{n} \sum_u \frac{1}{q_u} \sum_h R^2_{uh} - \frac{1}{n^2} \sum_u \frac{1}{q_u} Z^2_u - \text{SSR}$
	Error	$(qn-2m)(n-1)$	SSE	TSS − SSS − SSP − SSC − SSD − SSR − SSDB − SSRB

The analysis is based on the least squares fit of a model in which the performance of a given cow in a given period (Y_{ijk}) is equated to the sum of the following quantities: m = the general mean, g_i = the effect of the square $\left(\sum_i g_i = 0\right)$, p_{ik} = the effect of the period in that square $\left(\sum_k p_{ik} = 0\right)$, c_{ij} = the effect of the cow $\left(\sum_j c_{ij} = 0\right)$, $d_{h(ijk)}$ = the direct effect of treatment $\left(\sum_h d_{h(ijk)} = 0\right)$, $r_{h(ij,\,k-1)}$ = the residual effect of immediately preceding treatment $(r_{h(ijo)} = 0)$ and $\left(\sum_h r_{h(ij,\,k-1)} = 0\right)$, and e_{ijk} = a random error. For blocked designs, additional quantities were added to the model, namely, constants for blocks and for the interactions of block with direct and residual effects of treatment.

Estimates of the treatment means and their variances. If the design is not blocked, or if blocked and the interactions of treatment effects with blocks prove to be negligible, presentation of the over-all treatment effects is adequate.

The estimates of the over-all direct effect of the h^{th} treatment, d_h, and of the variance of the difference between two direct effects are given, respectively, by

$$\frac{W_h}{qn\,(n+2)} \qquad \text{and} \qquad \frac{2\,(n+1)}{qn\,(n+2)}s^2$$

where s^2 is the error mean square obtained from Table 1. The estimates of the over-all residual effect of the h^{th} treatment, r_h, and of the variance of the difference between two residual effects are obtained, respectively, as

$$\frac{nR_h - Z}{qn^2} \qquad \text{and} \qquad \frac{2}{qn}s^2.$$

The d's for the several treatments sum to zero, as do the r's. Instead of effects which sum to zero, it is customary, however, to publish treatment means which average to the grand mean performance over the whole experiment, $\overline{\overline{Y}}$. One might, therefore, wish to add $\overline{\overline{Y}}$ to the d's and r's. There seems to be little point, however, in adding $\overline{\overline{Y}}$ to the residual effects. Adding $\overline{\overline{Y}}$ to the direct effects is clearly appropriate if the residual effects prove to be negligible. If the residual effects are important, however, it would often seem appropriate to compute the sum of the direct and the residual effects. This sum will be called the "permanent" effect. In dairy practice, diets are not usually changed every few weeks as is done in the change-over trial. Instead, a given ration may be fed for several months. Thus, the permanent effects would appear to provide better estimates of treatment differences for practice than would the direct effects. The estimates of the permanent effect of the h^{th} treatment, t_h, and of the variance of the difference between two permanent effects are, respectively,

$$d_h + r_h \qquad \text{and} \qquad \frac{2\,(2n+3)}{qn\,(n+2)}s^2.$$

It is appropriate, of course, to add $\overline{\overline{Y}}$ to the t's.

If the design is blocked and the block by treatment interactions proves to be important, estimates of the various effects will be of interest for each block. The formulae just given are then modified by substituting W_{uh}, R_{uh}, Z_u, and q_u for W_h, R_h, Z, and q and computing the estimated effects and variances for each block. In place of $\overline{\overline{Y}}$, one will use $\overline{\overline{Y}}_u$ to obtain treatment means for each block. Estimated variances obtained are proper for comparisons of treatments only within blocks and not between blocks.

Missing values. In the event that an observation is missing or abnormal, it is necessary to substitute a value before applying the formulas given for the analysis of the data. The formula that provides a value minimizing the error sum of squares is formidable in appearance, but it is easy to use. Some X values are first computed, one for each treatment, as follows:

$$X_h = (n+1)x_{1h} - x_{2h} - 1$$

in which

$$x_{1h} = \begin{cases} 1 \text{ for the treatment on which the missing value occurs} \\ 0 \text{ for the other treatments} \end{cases}$$

$$x_{2h} = \begin{cases} 1 \text{ for the treatment occurring or supposed to have occurred last} \\ \quad \text{on the animal on which the missing value occurs} \\ 0 \text{ for other treatments} \end{cases}$$

Note that $\sum_h X_h = 0$. The missing value is computed as follows:

$$\frac{qn(n+2)\left[nC'_{ss} - G'_s + (n+1)P'_{ss}\right] + n\sum_h X_h W'_h + (n+1)(n+2)\left[nR'_s - Z'\right]}{qn^2(n-1)(n+2) - n\sum_h X^2_h - (n-1)(n+1)(n+2)}$$

in which

q and n are defined as previously

C'_{ss} = the sum of observations for the animal on which the missing value occurs

G'_s = the total for the square in which the missing value occurs

P'_{ss} = the sum for the period in which the missing value occurs for the square in which the missing value occurs

W'_h = the W values

R'_s = sum of all observations following the treatment which immediately precedes the missing value

Z' = the sum of all observations except those occurring in the first period

The quantities just defined are primed, to indicate that they are computed with the missing or abnormal value set at zero. They differ, therefore, from the corresponding quantities used in the final analysis.

If the design is blocked, the formula is applied using only the data from the block in which the missing value occurs. That is, the formula is applied as if that block were the whole experiment.

The formula was derived under the assumption that the observation immediately following the missing one contains the appropriate residual effect. This assumption will be adequate if residual effects are not large. Otherwise, one may wish to assume that both the missing value and the one following it are missing. The usual iterative procedure for applying missing-value formulas may then be employed.

If residual effects are truly zero, as they always are in the first experimental period, the missing-value formula simplifies somewhat, in that the last term of the numerator, $(n+1)(n+2)\left[nR'_s - Z'\right]$, and the last term of the denominator, $(n-1)(n+1)(n+2)$, do not appear. Use of the simplified formula for observations other than those in the first period may be justified if the investigator is reasonably certain that residual effects are negligible. Otherwise, the complete formula should be used. Actually, the difference in computational labor is minor.

NUMERICAL EXAMPLE

Data and preliminary computations. Since no trials using this type of design have been completed, computations will be made on uniformity data. These data are shown (Table 2), and are arranged to simulate an experiment comparing

TABLE 2

The data. Average daily FCM production in pounds. Treatments indicated in parentheses

Block	Square	Period	Individual data			Sum
			Cow 1	*Cow 2*	*Cow 3*	
		1	(1) 38.66	(2) 48.85	(3) 34.64	122.15
		2	(2) 37.43	(3) 46.88	(1) 32.27	116.58
	1	3	(3) 34.39	(1) 41.99	(2) 28.50	104.88
		4	(3) 31.30	(1) 39.61	(2) 27.13	98.04
		Sum	141.78	177.33	122.54	441.65
1			*Cow 4*	*Cow 5*	*Cow 6*	
		1	(1) 35.19	(2) 32.90	(3) 30.40	98.49
		2	(3) 33.50	(1) 33.12	(2) 29.50	96.12
	2	3	(2) 28.41	(3) 27.52	(1) 26.70	82.63
		4	(2) 25.12	(3) 25.10	(1) 23.09	73.31
		Sum	122.22	118.64	109.69	350.55
			Cow 7	*Cow 8*	*Cow 9*	
		1	(1) 25.72	(2) 30.80	(3) 25.35	81.87
		2	(2) 26.13	(3) 29.29	(1) 26.00	81.42
	3	3	(3) 23.35	(1) 26.41	(2) 23.86	73.62
		4	(3) 18.69	(1) 23.16	(2) 19.92	61.77
		Sum	93.89	109.66	95.13	298.68
2			*Cow 10*	*Cow 11*	*Cow 12*	
		1	(1) 21.80	(2) 21.37	(3) 22.84	66.01
		2	(3) 23.91	(1) 21.97	(2) 20.97	66.85
	4	3	(2) 21.69	(3) 19.38	(1) 18.59	59.66
		4	(2) 17.55	(3) 16.57	(1) 16.10	50.22
		Sum	84.95	79.29	78.50	242.74

	Total	Total less sum of first period
Block 1	792.20	571.56
Block 2	541.42	393.54
Over-all	1333.62	965.10

three treatments using a duplication of the basic design. The data are average daily production of FCM in pounds during periods five weeks in length. Treatments are indicated by the numbers in parentheses. Cows were allotted to squares on the basis of initial performance (not shown), as outlined earlier. For illustratory purposes, it is assumed that the two squares of higher producers form one block and the other two squares a second block.

Sums for the individual cows, periods, and squares are shown along with the data in Table 2. Also given are the block and grand totals, and these totals less the corresponding first-period sums. Additional sums and preliminary quantities computed therefrom are presented (Table 3).

TABLE 3

Additional sums and preliminary quantities computed from the data and sums in Table 2

Block	Treatment	Sum of observations on treatment D_h	Sum of observations following treatment R_h	Sum of totals for cows receiving treatment last L_h	W-values W_h
1	1	270.63[a]	189.65[a]	287.02[a]	3.30[a]
	2	257.84	193.34	244.76	−5.60
	3	263.73	188.57	260.42	2.30
	Sum	792.20	571.56	792.20	0
2	1	179.75	132.54	188.16	−10.58
	2	182.29	130.67	180.08	7.66
	3	179.38	130.33	173.18	2.92
	Sum	541.42	393.54	541.42	0
Over-all	1	450.38	322.19	475.18	−7.28
	2	440.13	324.01	424.84	2.06
	3	443.11	318.90	433.60	5.22
	Sum	1333.62	965.10	1333.62	0

[a] $270.63 = 38.66 + 41.99 + 39.61 + \ldots + 23.09$.
$189.65 = 37.43 + 39.61 + 28.50 + \ldots + 23.09$.
$287.02 = 177.33 + 109.69$.
$3.30 = (3+1)(270.63) - 287.02 - 792.20$.

Analysis of variance. The computations proceed as follows:

Correction factor:

$$\frac{(1333.62)^2}{4(3)(3+1)} = 37{,}052.9647$$

Total sum of squares:

$$(38.66)^2 + (48.85)^2 + \ldots + (16.10)^2 - 37{,}052.9647 = 2{,}661.6965$$

Squares sum of squares:

$$\frac{1}{3\,(3+1)}\left\{(441.65)^2+(350.55)^2+(298.68)^2+(242.74)^2\right\}-37{,}052.9647 = 1{,}786.4082$$

Periods within squares sum of squares:

$$\frac{1}{3}\left\{(122.15)^2+(116.58)^2+\ldots+(50.22)^2\right\}-1{,}786.4082-37{,}052.9647 = 407.1403$$

Animals within squares sum of squares:

$$\frac{1}{3+1}\left\{(141.78)^2+(177.33)^2+\ldots+(78.50)^2\right\}-1{,}786.4082-37{,}052.9647 = 451.7881$$

Direct effects sum of squares:

$$\frac{1}{4(3)(3+1)(3+2)}\left\{(-7.28)^2+(2.06)^2+(5.22)^2\right\} = 0.3520$$

Residual effects sum of squares:

$$\frac{1}{4(3)}\{(322.19)^2+(324.01)^2+(318.90)^2\}-\frac{(965.10)^2}{4(3)(3)}=1.1180$$

Direct effects by blocks sum of squares:

$$\frac{1}{2(3)(4)(5)}\{(3.30)^2+(-5.60)^2+\ldots+(2.92)^2\}-0.3520=1.5370$$

Residual effects by blocks sum of squares:

$$\frac{1}{2(3)}\{(189.65)^2+(193.34)^2+\ldots+(130.33)^2\}$$
$$-\frac{1}{2(3)(3)}\{(571.56)^2+(393.54)^2\}-1.1180=1.4393$$

Error sum of squares:

$$2{,}661.6965 = 1{,}786.4082 - \ldots - 1.4393 = 11.9136$$

These results, along with the corresponding degrees of freedom and other computations of the analysis of variance, are given (Table 4).

TABLE 4

Summary of the analysis of variance of the uniformity data

Variance source	Degrees of freedom	Sum of squares	Mean square	F
Total	47	2661.6965		
Squares	3	1786.4082		
Periods within squares	12	407.1403		
Animals within squares	8	451.7881		
Direct effects	2	0.3520	0.1760	0.24
Residual effects	2	1.1180	0.5590	0.75
Direct effects by blocks	2	1.5370	0.7685	1.03
Residual effects by blocks	2	1.4393	0.7197	0.97
Error	16	11.9136	0.7446	

As would be expected in uniformity data, the direct and residual effects of treatment and their interactions with blocks are not significant, these several mean squares being about the same magnitude as the error mean square.

If the design had not been blocked, the treatment by block interactions would not have been computed. This would have yielded an error sum of squares of 14.4709 with 20 degrees of freedom, or an error mean square of 0.7236.

Treatment means and variances. Estimates of the treatment means and the variances of treatment differences are shown (Table 5) for each block and over-all, along with the block and over-all mean performances. Example computations are:

Direct effect: treatment 1, block 1

$$\text{Mean} = \frac{3.30}{2(3)(5)} + 33.01 = 33.12$$

$$\text{Variance of a difference} = \frac{2(3+1)}{2(3)(3+2)}(0.7446) = 0.1986$$

Residual effect: treatment 3, block 2

$$\text{Mean} = \frac{3(130.33) - 393.54}{2(3)(3)} = 0.14$$

$$\text{Variance of a difference} = \frac{2(0.7446)}{2(3)} = 0.2482$$

Permanent effect: treatment 1, over-all

$$\text{Mean} = 27.66 + 0.04 = 27.70$$

$$\text{Variance of a difference} = 0.0992 + 0.1240 = 0.2234$$

COMMENTS

Expressed as a coefficient of variation, the experimental error in these uniformity data was

$$\frac{100\sqrt{0.7446}}{27.78} = 3.1\%.$$

TABLE 5

Estimated treatment means and their variances—uniformity data

Block	Treatment	Direct effect (block or over-all mean added)	Residual effect	Permanent effect (block or over-all mean added)
1	1	33.12	−0.14	32.98
	2	32.82	0.47	33.29
	3	33.09	−0.33	32.76
	Mean	33.01	0	33.01
	Variance[a]	0.1986	0.2482	0.4468
2	1	22.21	0.23	22.44
	2	22.81	−0.09	22.72
	3	22.66	−0.14	22.52
	Mean	22.56	0	22.56
	Variance[a]	0.1986	0.2482	0.4468
Over-all	1	27.66	0.04	27.70
	2	27.81	0.19	28.00
	3	27.87	−0.23	27.64
	Mean	27.78	0	27.78
	Variance[a]	0.0992	0.1240	0.2234

[a] Variance of a treatment difference. The standard error of a difference between two treatments is given by the square root of this variance.

In the author's experience, this error is near the low extreme for change-over trials of the Latin-square or rotational type with dairy cattle. In 18 Latin-square trials examined, the errors ranged between 1.4 and 9.4%, with an average between 5 and 6%. These figures agree fairly well with those obtained by Taylor and Armstrong (*5*).

Earlier in this paper, some comments were made regarding the advantages and disadvantages of the extra-period designs as compared to the regular Latin-squares. It was also pointed out that the switch-back trials may be preferable to either of these, if residual effects can be assumed to be negligible. Principal factors to be considered in making a choice among these three types of designs seem to be, (*a*) the relative sizes of the experimental errors per observation, (*b*) the effective replication per observation for the direct, the residual, and the "permanent" effects, (*c*) whether residual effects are present or not, and (*d*) whether or not the investigator is particularly interested in assessing the residual and the "permanent" effects, or is simply interested in demonstrating treatment differences without regard to residual effects. These questions have not been studied adequately enough to give a refined basis for choice of design. Error estimates obtained by Taylor and Armstrong (*5*), together with error estimates obtained by the author (unpublished), indicate that the experimental errors per observation in dairy-cow experiments generally are the lowest with the switch-back trials, and are somewhat higher for the extra-period designs than for the Latin-squares. From a consideration of these results, together with the effective replication per observation, it appears certain that the extra-period designs are particularly advantageous if there is particular interest in residual and permanent effects and the number of treatments does not exceed four or five.

If interest lies especially in "permanent" effects, a question may be raised as to whether continuous trials with covariance on initial production might not be superior to change-over trials, even when special provision is made to estimate "permanent" effects, as in the extra-period Latin-square. Unpublished studies, as yet incomplete, indicate no advantage for the continuous trials when residual effects do not last more than one period.

Since the useful portion of the lactation curve is limited, it seems pertinent to point out that the duration of the extra-period trial need not be longer than that of the corresponding Latin-square. For example, if a Latin-square trial were planned to require four 6-week periods, the extra-period design could be made to consist of five 5-week periods.

SUMMARY

Latin-square change-over trials balanced for carry-over effects have been modified by adding an extra period of observation. During this extra period the treatment pattern is identical with that in the last period of the Latin-square design. This modification reduces somewhat the information per observation about treatments if residual effects do not exist, but increases the information on residual effects and especially on "permanent" effects of treatment. Use of the extra-period designs is discussed and patterns for two to five treatments are

given. The statistical analysis is outlined symbolically and is illustrated numerically with uniformity data.

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EFFECTS OF VARIOUS HAY:CONCENTRATE RATIOS ON NUTRIENT UTILIZATION AND PRODUCTION RESPONSES OF DAIRY COWS. II. OBSERVATIONS ON RATION DIGESTIBILITY AND ON THE EXCRETION PATTERN OF CHROMIC OXIDE[1]

S. BLOOM,[2] N. L. JACOBSON, R. S. ALLEN, L. D. McGILLIARD,[3] AND P. G. HOMEYER
Departments of Animal Husbandry, Chemistry, and Statistics
Iowa Agricultural Experiment Station, Ames

The relationship between digestibility of feed and efficiency of milk production with Holsteins was investigated. Neither genetic ability nor feeding level significantly affected digestibility. It is believed that animals vary in their efficiency to utilize nutrients after absorption, but that differences in digestion and absorption are less important. *Editor.*

A number of investigators *(6, 12, 30)* have reported that efficiency of digestion does not vary greatly among cattle. However, Jarl *(17)* reported slight variation in the digestive powers of cows (3.5% difference between the most and least efficient cows) and Baker *et al. (3)* reported that beef steers which used the least feed per pound of gain seemed to be superior in their digestion of crude fiber.

This phase of the current study was designed primarily to determine digestibility values for the various rations described previously *(4)* and, thereby, to provide further information on the relationship between ration digestibility and efficiency of milk production. The large number of cows involved in this study and the facilities available precluded total fecal collections; therefore, the chromic oxide indicator method was employed. A number of reports *(7, 13, 14, 20, 27)* have provided data that stress the importance of collecting fecal grab samples at specified times of the day, if reliable estimates of ration digestibility, or of total fecal output in the case of grazing animals, are to be obtained. The secondary objective of this work was to study the fecal excretion pattern of chromic oxide under the wide range of dietaries imposed.

EXPERIMENTAL PROCEDURE

A digestion trial was run on each of 36 cows, described in the experimental design in the preceding paper *(4)*, approximately four to five months after

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[2] Present address: Department of Animal Husbandry, Agricultural Research Station, Rohovot, Israel.

[3] Present address: Dairy Department, Michigan State University, East Lansing.

each cow started the experimental period. Since the cows entered the experiment over a wide time interval, they were placed in eight convenient groups varying in size from three to seven cows each. Digestion trials for animals within each group were run simultaneously. During the digestion trial, the daily feed allowance, which had been determined at the start of the trial, was continued at a constant rate for a 10-day preliminary and a three-day collection period. When necessary to insure complete consumption, high hay or high concentrate rations were adjusted downward in amounts to maintain the particular hay-to-concentrate ratio. The regular weekly adjustments in feeding implicit in the experimental design were discontinued during these trials, and necessary adjustments in feeding to conform to the regular feeding schedule were made at the end of the digestion trial.

During the three-day collection period, nine fecal samples were obtained from each cow, usually by stimulation of defecation, according to the following schedule: first and third days—9 A.M. and 5 P.M.; second day—5 A.M., 9 A.M., 1 P.M., 5 P.M., and 9 P.M. Three 250-g. portions of each sample were immediately and directly weighed into polyethylene plastic bags, sealed, packed in cardboard containers, and placed in a freezer. All samples were stored in the frozen state until analyzed.

Chromic oxide, the digestibility indicator employed, was prepared for feeding according to the procedure of Kane *et al. (19)*, except that leavening was added before baking, drying, and subsequent grinding of the material. The chromic oxide–flour mixture was incorporated into a premix with the concentrate mixture. The premix was so formulated that each pound contained approximately 15 g. of chromic oxide. Before each digestion trial and for each cow, 13 one-pound portions of the chromic oxide–flour-concentrate premix were weighed into paper bags. During the digestion trial, one bag of the premix was incorporated into each total daily concentrate allowance, from which one pound had been deducted to allow for the pound of premix. This mixture was fed in four equal portions at 7 A.M., 4 P.M., 7 P.M., and 4 A.M.

During each trial one-per cent aliquots of the total hay and concentrates fed each day for the last six days of the 13-day digestion trial were composited and sampled. Proximate analyses of the feeds and feces were conducted as described by the A.O.A.C. *(2)*, except that a Goldfisch Extractor was used for estimation of ether extracts. Chromic oxide in the feces was determined by the colorimetric method described by Schürch *et al. (26)*. Data on the chemical composition of the feeds used and the cows involved in each of the eight digestion trials are given (Table 1).

RESULTS

Results of the digestion study are shown (Table 2), where the data have been summarized as follows: Each entry is the average of the analyses of the 27 fecal samples, nine for each of three cows at each feeding level and at each ability group. These data are presented for each hay-to-concentrate feeding

TABLE 1
Proximate analysis of the feeds in each digestion trial

Sample[a]	Nutrients					
	Prot.	E.E.	C.F.	Ash	Moist.	N.F.E.
	%					
Concentrates						
I	15.8	2.9	6.5	5.2	11.0	58.6
II	18.8	2.5	8.8	8.2	12.8	48.9
III	17.2	2.2	9.0	7.1	10.7	53.8
IV	20.8	1.8	8.6	6.8	12.5	49.5
V	20.4	2.9	8.7	5.8	11.3	50.9
VI	18.5	3.0	9.9	5.6	10.9	52.1
VII	20.8	2.5	7.9	6.2	10.6	52.0
VIII	19.7	2.7	9.1	4.9	11.8	51.8
Hay						
I	11.6	1.3	33.6	7.9	7.6	38.0
II	14.2	2.0	22.9	7.6	10.4	42.9
III	14.8	2.1	33.7	8.2	9.5	31.7
IV	12.1	2.0	36.5	7.8	10.9	30.7
V	11.8	1.7	36.6	7.3	11.8	30.8
VI	12.2	1.6	40.8	7.8	10.2	27.4
VII	10.2	1.5	43.1	7.0	10.6	27.6
VIII	13.3	1.6	37.8	7.1	11.2	29.0

[a] Cow groups corresponding to the various samples were as follows: I–3529, 3516, 2710, 3538, 3266, 2378; II–2553, 2392, 2159; III–2643, 3302, 3440; IV–3160, 3128, 2600; V–3444, 3469, 3432, 3174, 3263; VI–2982, 3294, 3157, 2963, 3450; VII–3291, 2606, 3439, 2976; VIII–3142, 3483, 3493, 3597, 3632, 2649, 3272.

ratio. The average for ration is that of all 81 samples from the nine cows on each ration. Table 3 presents the final combination of the data on the basis of the 12 cows at each feeding level and at each ability level, and then presents the over-all digestibility of each feed constituent for the 36 cows.

An analysis of variance was computed for the data on digestibility and on the fecal excretion pattern of the chromic oxide. Analysis of each feed constituent (Table 4), brought within its scope five sources of variance in the data, namely, ration, level of feeding, ability, day of collection, and time of day at which the collection was made. Only the fecal collections made at 9 A.M. and 5 P.M. for each of the three days of the digestion trial were used in these analyses. The estimate of error for tests of significance in each analysis of variance is the weighted average of the interactions with days. For the few times when an individual fecal sample could not be obtained, values for the missing data were calculated according to the method of Snedecor *(28)*.

Of the three major factors, ration, level of feeding, and ability, the widely varying rations had the greatest effect upon dry matter digestibility (Table 4). From the high to the low hay rations, digestibility coefficients were, respectively, 54.5, 58.5, 55.2, and 63.1%. The nine cows at the 35:65 hay-to-concentrate ratio showed a lowering of dry matter digestibility against the general rise, as the proportion of hay in the rations was decreased. Level of feeding and ability exerted no appreciable influence.

Digestibility of protein ($N \times 6.25$) generally followed a pattern similar to that for dry matter, across the four hay-to-concentrate ratios, and showed

TABLE 2

Digestion coefficients averaged as to level of feeding, producing ability, and ration[a]

Hay-to-conc. ratio	Level of feeding			Ability			Av. for ration
	High	Medium	Low	High	Medium	Low	
	%						
H-75:C-25							
D.M.	54.5	51.4	57.7	53.2	55.5	54.8	54.5
Prot.	57.8	50.7	63.5	54.5	56.6	60.9	57.3
E.E.	11.9	9.3	15.3	12.5	5.2	18.8	12.2
C.F.	23.5	35.7	45.5	28.7	36.7	39.3	34.9
N.F.E.	65.4	58.9	61.1	63.3	61.4	60.7	61.8
H-55:C-45							
D.M.	57.5	61.6	56.3	57.1	59.7	58.7	58.5
Prot.	63.9	66.6	63.9	61.4	67.6	65.4	64.8
E.E.	31.0	45.8	23.4	26.5	38.6	35.2	33.4
C.F.	37.2	46.4	40.8	43.6	40.8	40.1	41.5
N.F.E.	61.4	65.9	60.3	60.3	63.7	63.6	62.5
H-35:C-65							
D.M.	58.3	55.9	51.5	58.8	55.4	51.4	55.2
Prot.	58.2	59.9	62.3	60.6	60.1	59.8	60.2
E.E.	37.5	35.0	31.4	38.3	34.7	30.8	34.6
C.F.	31.0	24.1	26.0	34.1	25.6	21.4	27.0
N.F.E.	67.6	65.2	55.5	67.0	63.3	58.0	62.8
H-15:C-85							
D.M.	60.2	62.1	67.1	63.8	61.8	63.8	63.1
Prot.	69.3	72.8	75.6	74.0	76.5	67.2	72.6
E.E.	49.8	54.6	58.8	60.7	48.5	54.0	54.4
C.F.	19.9	13.6	28.7	28.4	16.9	16.8	20.7
N.F.E.	65.3	68.8	72.4	67.5	66.2	72.8	68.8

[a] Each of the levels of feeding and producing ability coefficients is an average of three cows; each ration coefficient is an average of nine cows.

a similar depression in digestibility at the 35:65 ratio. Differences in protein digestibility due to levels of feeding and of ability were not significant.

Average digestion coefficients for ether extract increased from 12.2% on the high ration to 33.4, 34.6, and 54.4%, respectively, as the hay in the ration decreased. As with the results presented to this point, the fairly orderly increase in ether extract digestibility was interrupted at the 35:65 hay-to-concentrate ratio, where it remained at about the same level as at the 55:45 feeding ratio. As was observed for dry matter and protein, levels of feeding and of ability had no significant effect upon ether extract digestibility.

TABLE 3

Summary of mean digestibility coefficients

Level of feeding	Ability	D.M.	Prot.	E.E.	C.F.	N.F.E.
		%				
High		57.6	62.3	32.6	27.9	64.9
Medium		57.8	62.5	36.2	30.0	64.7
Low		58.2	66.4	32.2	35.3	62.3
	High	58.2	62.6	34.5	33.7	64.5
	Medium	58.1	65.2	31.7	30.0	63.7
	Low	57.2	63.3	34.7	29.4	63.8
Over-all average		57.8	63.7	33.6	31.0	64.0

TABLE 4
Analysis of variance for digestibility data

Source of variation	Degrees of freedom	Mean squares				
		D.M.	Prot.	E.E.	C.F.	N.F.E.
Ration (R)	3	816.84[a]	2425.37[b]	15288.21[b]	4332.84[a]	636.94
Level (L)	2	9.21	228.96	610.30	709.13	240.40
Ability (A)	2	9.77	59.08	590.44	420.92	32.09
RL	6	344.51	326.50	1131.46	1127.48	554.87
Error (a)	22	194.69	310.69	652.52	997.47	272.39
Days (D)	2	88.38	20.21	287.21	462.78[a]	48.31
Time of day (T)	1	1311.18[b]	1227.70[b]	4606.60[b]	4310.87[b]	673.14[b]
TR	3	17.79	64.43	8.80	157.78	35.63
TL	2	41.38	45.28	145.02	33.79	100.50
TA	2	42.15	19.66	9.59	120.05	67.83
TRL	6	36.65	42.17	178.48	107.89	45.81
TRA	6	8.75	12.37	145.17	40.18	22.62
TLA	4	12.74	11.34	19.63	103.71	19.45
RLAT	12	39.12	22.22	118.11	175.34	59.97
Error (b)	142	34.45	24.27	185.62	121.68	33.75
Total	215					

[a] $P < 0.05$.
[b] $P < 0.01$.

Effect of ration upon crude fiber digestibility was significant ($P < 0.05$) and the values from the high to the low hay rations were, respectively, 34.9, 41.5, 27.0, and 20.7%. Although the effect of level of feeding was not significant, there was a trend for greater crude fiber digestibility by the cows at the low level of feeding. Effect of ability upon crude fiber digestion was not significant, but at three of the four hay-to-concentrate ratios (Table 2) animals of highest ability had the highest crude fiber digestibility coefficients, with a reversal of this pattern occurring at the highest hay ration.

A plausible explanation for the above reversal at the highest hay ration may be as follows: The three animals of low ability on the high hay ration generally consumed smaller total amounts of hay than the high- and medium-ability animals on the same ratio of feeds. The greatest total consumption of hay seemed related to a lowered crude fiber digestibility. Possibly this condition may have been due to the more rapid passage of feed through the digestive tract and, consequently, to the shorter time for bacterial action in the rumen. This relationship of high hay intake and lowered crude fiber digestibility was supported by cows No. 2553 and No. 2392 (high- and medium-ability cows, respectively, at the high level of feeding). These cows consumed the greatest amounts of hay during the digestibility trial and had the lowest crude fiber digestion coefficients of any of the nine cows on this ration.

From the high hay to the low hay rations, the nitrogen-free extract digestion coefficients were, respectively, 61.8, 62.5, 62.8, and 68.8%. Effects of ration, level of feeding, and ability were nonsignificant. Only at the highest concentrate ratio (15:85), in which the greatest proportion of the nutrients was in the form of NFE, did this fraction increase in its digestibility percentage from the high to the low level of feeding. The 35:65 hay-to-concentrate ratio interrupted the generally ascending NFE values from the high to the low hay rations.

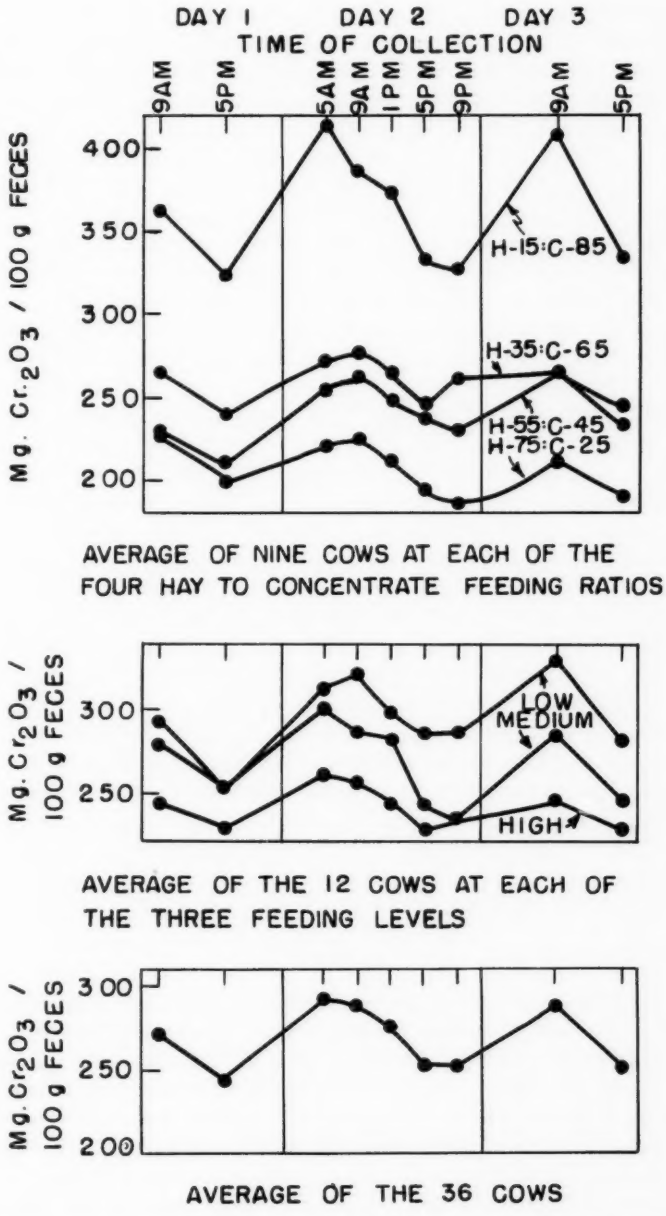

FIG. 1. Summary of fecal excretion patterns of chromic oxide over the three-day collection period.

This ratio had an NFE digestibility value almost equal to that of the 55:45 ratio, although the total amount of the components considered in the NFE fraction had increased considerably, in moving from a ratio of 2 pounds of hay to one pound of concentrate (55:45) to a ratio of one pound of hay to one pound of concentrate (35:65).

Patterns of fecal excretion of chromic oxide (shown Figure 1) are presented successively on the basis of the average curves for each of the four hay-to-concentrate ratios, those for each of the three feeding levels, and finally, that for all 36 cows in this study. For more than two-thirds of the cows, the maximum excretion of chromic oxide was observed in samples at 5 A.M. or 9 A.M.; whereas, the minimum excretion was observed in samples at 5 P.M. or 9 P.M. The orderly decrease in excretion from 5 A.M. to 9 P.M. is shown by the descending curve during the second day of the trial, when more frequent collections were made. Digestibility data for each feed constituent used in the statistical analyses were functions of the amount of chromic oxide present in the feeds and, subsequently, in the feces. Analyses showed that the effect of collection time upon digestibility of the nutrients was significant ($P < 0.01$).

The excretion pattern of chromic oxide was quite consistent from day to day, especially when all animals are considered (Figure 1). This was substantiated by the statistical analyses in which, for example, the mean square for the effect of the day of collection (D) upon dry matter digestibility was one-fifteenth as great as that for the time of collection. This indicated that the chromic oxide had been fed in a uniform manner, that it had been thoroughly distributed in the ingesta and that, over the collection period, it had been excreted uniformly from day to day.

The variability in chromic oxide excretion increased from the high to the low hay rations and from the high to the low levels of feeding (Figure 1). Thus, there appears to be a definite relationship between the amount and type of gastrointestinal fill and the degree of variability found in the chromic oxide content of individual collections. Despite this increasing variability among individual collections, under the above cited conditions, the typical daily excretion pattern was apparent.

DISCUSSION

These data indicated that neither the ability (genetic effect) nor the feeding level exerted important influences upon the powers of digestion. Thus, the observed differences in milk production among the cows of different producing abilities on similar rations, as previously reported *(4)*, can not be explained on the basis of differences in digestibility. A possible explanation of these results was found in the work of Armsby *(1)* and Mumford *et al. (25)*, who showed that, although a probable error of three to four per cent was common in the determination of apparent digestibility, there were cases in which the variation became as high as eight to nine per cent among cattle receiving the same test ration. In this study, samples were taken for a comparatively short period,

and if more frequent collections had been made, the differences might have been much smaller for cows of different abilities on identical rations. Nevertheless, the average results in this work revealed a total difference in dry matter digestibility of but one per cent in favor of the high-ability cows and of but one-half of one per cent in favor of the cows at the low level of feeding (Table 3).

Other studies approaching the pattern of this report, though with different experimental designs and other goals, support the current findings. Hansson *(12)*, in digestibility studies with monozygous cattle twins fed at four levels, found that the total difference in organic matter digestibility over these levels was one per cent in favor of the high-fed twins and that there were no larger differences between individual twin pairs. Watson *(30)*, in a summarization of 15 years of experimentation with various feeding levels, also stated that the levels had no effect upon digestibility or, where differences existed, they were of small magnitude. Hansson *(12)* also indicated that heredity did not markedly affect the animals' capacity to digest the feed.

Brumby and Hancock *(5)*, in a recent study of the galactopoietic role of growth hormone in dairy cattle, found that when daily subcutaneous injections of growth hormone were administered to identical-twin cattle there was a marked increase in milk and butterfat production, with an apparent increase in the efficiency of production. Digestibility studies yielded almost identical digestion coefficients for both the treated and untreated twins, which eliminated this possible explanation of the observed increase in efficiency of production. Baker *et al. (3)* found wide differences in the efficiency of feed utilization in beef cattle, but small differences in their efficiencies of digestion. The current work and the above supporting evidence lead to the postulation that differences among animals exist in the efficiency of utilization of nutrients after absorption, but that differences in digestion and absorption of nutrients probably are of lesser magnitude.

The only indication, although not statistically significant, of the relationship of animal ability to the digestion of nutrients in these data was observed with crude fiber (Table 3). Baker *et al. (3)* demonstrated a similar superiority in crude fiber digestion in beef steers of high feed efficiency. These workers reported a total range of difference of 4.9% between steers of low and high efficiency, which compared closely with the 4.3% difference in crude fiber digestion between low- and high-ability cows in the present work. This indicates that in digestibility studies, crude fiber digestion may be a factor related to productive efficiency. Further study is indicated, in order to elucidate this relationship.

While the total energy fed was held constant at each hay-to-grain ratio, the dry matter digestibility increased from high to the low hay rations with an interruption of this trend at the 35:65 hay-to-concentrate ratio, in which the two feeds were offered in approximately equal proportions by weight. This depression of dry matter digestibility perhaps may best be explained by combining the results of the literature, relative to proportions of nutrients in the

ruminant ration and to bacteriological studies on various combinations of hay and readily available carbohydrates. Addition of starch, sugar, and similar substances may cause a depression in the digestibility of the materials with which they are fed *(11, 23, 29)*.

Swift *et al. (29)* and Hamilton *(11)* related the decreased digestibility to the preference of the rumen microorganisms for the simpler carbohydrates. In digestion studies with sheep, wherein the proportions of hay and concentrates approached most closely the 35:65 hay-to-concentrate ratio in this study, Lindsey and Smith *(21)* reported an average decrease in ration digestibility of eight per cent, and Louw and van der Wath *(22)* observed a nine per cent decrease in cellulose digestion when maize was added in nearly equal proportions to a poor veld hay. Bacteriological studies by these workers *(22)* showed that small amounts of maize and meatmeal added to veld hay favored the growth of rumen organisms; whereas, almost equal amounts of hay and maize tended to reduce the number of organisms in the ruminant. Gall *et al. (9)* found, with cattle on practical farm rations, that a high grain ration (proportions of hay and grain not indicated) stimulated an increase in types of organisms already present in moderate numbers in the rumen, rather than encouraged development of different types of bacteria. Later work by Gall *et al. (10)*, employing sheep on purified rations which closely approached the 15:85 hay-to-concentrate ration in this work, showed that cultures of rumen flora consisted almost entirely of fast-growing organisms. Tests for the presence of slower-growing organisms, usually associated with cellulose digestion, were largely negative.

In applying these previous observations to the results of the present study, it can be postulated that on the high concentrate ration (15:85) the microorganisms had adjusted their types to the great predominance of available carbohydrates and that the 55:45 ratio (approximately two pounds of hay to one pound of concentrates) stimulated development of microorganisms capable of greater roughage use. The 35:65 hay-to-concentrate ratio, however, may have represented an unfavorable zone of nutrient combination for satisfactory microorganism activity upon, and perhaps adjustment to, available substrates. At this ratio the microorganisms were presented with sufficient available carbohydrates to decrease their attack upon the roughage in this ration, but with an insufficient amount to achieve their complete adjustment to the high concentrate type of ration.

The present work and the studies of Kane *et al. (20)* and Edin *(7)* have yielded daily chromic oxide fecal excretion curves remarkably similar in nature, despite differences in the mode of chromic oxide administration, time required to consume the ration containing the indicator, time intervals between feedings, length of the preliminary feeding period of the indicator, and the time span over which the trials were conducted. Hardison *et al. (13)* also observed a similar diurnal pattern when the chromic oxide was administered once daily by capsule. However, when the marker was given twice daily, the diurnal

variation was markedly reduced and the diurnal pattern was altered. On the other hand, an appreciable diurnal variation was evident in the present study, even though the marker was fed four times daily (in the concentrate mixture). In work with goats, Kameoka *et al. (18)* observed only one distinct peak in chromic oxide excretion when the marker was fed at 9 A.M. and 4 P.M.; whereas, two peaks were noted when feedings were at 12-hour intervals. These observations were at more frequent intervals than those in the present study, which may account, at least in part, for the apparent difference in chromic oxide excretion patterns.

Ewing and Smith *(8)* and Mumford *(24)* reported that the rate of feed passage became more rapid as the level of feeding was increased, and that the smaller and finely ground feeds passed through the animal more quickly than larger and more coarse feeds. The above data suggest that in the present experiment there were variations in the rate of passage of feed, owing to the type of feed and to the interaction of the levels of feeding and diverse physical natures of the rations fed. Despite these added variables, the observed maxima and minima of chromic oxide excretion occurred at similar time intervals.

Observations by Edin *(7)* suggested a correlation between the time of the greatest amounts of rumination and the subsequent maximum excretion of chromic oxide. In the current work, however, cows with depressed rumination on the high concentrate rations exhibited the same general time of the observed maximal excretion as those on the high hay rations. It is possible that the periodicity in the chromic oxide excretion pattern is difficult to alter. Some support for this hypothesis may be gained from the observations of Herring and Brody *(16)*, who found that the diurnal rhythm associated with the heat increment in feeding (SDA) in the rat could be extinguished only after a week of continuous feeding (one-eighth of the daily ration every three hours) and continuous light; whereas other measures, used singly or in combination, failed to disturb this diurnal rhythm.

Considering the many metabolic activities subject to a basic diurnal rhythmicity, these and the earlier observations on the nature of the chromic oxide excretion pattern suggested the following tentative explanation:

Although digestion in the ruminant is a continuous process, the absorption of nutrients may proceed at varying rates over a daily period. Such differential rates in absorption of the gut contents would then be reflected in the varying quantities of chromic oxide excreted over a daily period, in relation to the remaining residue in the tract. During periods of high absorptive activity, the amount of chromic oxide would increase in relation to the smaller relative amount of ingesta remaining in the tract, the reverse being the case during a period of low absorptive activity. This proposal, though tenuous, receives some credence from a report which presented evidence of diurnal rhythmicity in the digestive tract. In a study of the production of gastric juice in the human fasting stomach, Hellebrandt et al. *(15)* found that although secretion was continuous throughout a 24-hour period, the total acidity of gastric juice liberated spontaneously during sleep at night was higher than that produced in the daytime.

The bulk of evidence indicates that diurnal variation or a "periodicity" in the excretion of chromic oxide is to be anticipated when this indicator substance is used in digestibility trials. The indiscriminate adoption of time (s) for obtaining fecal samples is precluded in this type of investigation, and it is of interest that Edin *(7)* made this observation in the first reported use of this indicator with dairy cattle. This early work suggested, as have a number of more recent reports, that fecal samples should be taken at 12-hour intervals, or at those hours when the variation of the chromic oxide in the feces averages to a mean value for the daily period.

SUMMARY

A digestibility study made with the chromic oxide ratio technique was conducted with 36 dairy cows. Four ratios of hay to concentrates (replacement on an Estimated Net Energy basis), three feeding levels, and three ability levels were employed. The nature of the fecal excretion pattern of chromic oxide, under the wide range of conditions imposed by the experimental design, also was studied.

From the high to the low hay rations, the dry matter digestibilities were, respectively, 54.5, 58.5, 55.2, and 63.1%. Dry matter digestibilities for high, medium, and low ability cows were 58.2, 58.1, and 57.2%, respectively, and for high, medium, and low feeding levels were 57.6, 57.8, and 58.2%. Digestibility values for the individual nutrients also are reported.

In this study, the fecal excretion of chromic oxide followed a diurnal pattern. Maximum excretion of chromic oxide was observed in samples taken at 5 A. M. or 9 A. M. and the minimum excretion was observed in samples at 5 P. M. or 9 P. M. This diurnal excretion pattern was largely independent of the wide variation in the physical nature of the rations and of the feeding level.

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THE VALUE OF ALFALFA HAY AND ALFALFA SILAGE WHEN FED AS SUPPLEMENTS TO COWS ON PASTURE[1]

MAURICE COLE, D. M. SEATH, C. A. LASSITER,[2] AND JOE RUST
Dairy Section, University of Kentucky, Lexington

Alfalfa hay and alfalfa silage did not increase the milk production of cows on pasture, but both reduced the amount of dry matter obtained from pasture feeding. *Editor.*

Summer slumps in milk production are common in most states. Efforts to prevent or minimize this condition have followed various routes.

A limited amount of work has been done to determine the value of supplementing summer pastures with such feedstuffs as hay, silage, or an extra allotment of grain over and above that normally fed to milk cows. The results of these studies have been reviewed previously *(7)*.

During the summer of 1954 Seath *et al. (7)* fed cows grazing a Kentucky bluegrass pasture twice daily all the alfalfa hay they would consume. No improvement in milk production was noted, although they did observe significant gains in body weight as a result of hay feeding. Cows fed hay consumed significantly less dry matter from pasture than cows not fed hay.

Because of the lack of experimental data relating to the subject of supplementing summer pastures with barn feeding of additional roughages, this experiment was undertaken to measure what influences the feeding of alfalfa hay or alfalfa silage to cows on summer pasture would have on milk production, body weight, dry matter intake, and the digestibility of the ration, and to compare alfalfa hay with alfalfa silage as a supplement to summer pasture.

EXPERIMENTAL PROCEDURE

A total of 18 cows, nine Holsteins and nine Jerseys, were selected from the University dairy herd. They were sorted so as to have three groups as similar as possible, with regard to breed, milk production, and body weight. Group I served as the control animals, Group II was supplemented with alfalfa hay, and Group III received alfalfa silage as a supplement to the pasture.

During the period May 25 to September 14 the cows were allowed to graze a permanent pasture consisting primarily of Kentucky bluegrass with some white clover which decreased in amount as the season advanced. The condition of the

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[2] Present address: Michigan State University, East Lansing.

pasture varied from excellent to poor, as described by Morrison (*5*), with the latter condition prevailing during the late summer when the herbage was short and brown.

All three groups of cows received a grain ration containing 12.8% crude protein. The initial grain allotment for each cow was based on milk production and pasture quality as outlined by Morrison in table VIIIb of *Feeds and Feeding* (*5*). After this initial allotment, the grain ration was adjusted every 4 weeks based on the average percentage decline in production for all groups of cows as outlined by Lucas (*4*).

The six cows in Group II, which received alfalfa hay, were allowed all of the hay (equivalent in quality to U. S. No. 1) they would consume during two 2-hour periods (A.M. and P.M.) each day while in stanchions at milking time. This was also the manner in which Group III received the alfalfa silage. Accurate weights on all hay, silage, and grain consumed and refused were kept for each cow.

Digestibility trials and measures of dry matter intake were conducted on all cows every 2 weeks by combining the fecal chromogen(s) *(6)* and the chromic oxide (*3*) techniques. Each cow received daily 12 g. of chromic oxide in a gelatin capsule. Fecal grab samples were collected at 6:00 A.M. and 4:00 P.M. daily for the last 5 days of each 2-week test period. These samples were composited and analyzed for dry matter, chromogen, and chromic oxide. The feces were analyzed for chromic oxide by the method outlined by Gehrke and Becker (*2*), which is a modification of the method published by Bolin (*1*). Grass samples for dry matter content were gathered on the middle day of each test period. All feed refusals were weighed at the beginning and conclusion of each sampling period and at weekly intervals during the preliminary periods. All refusals were analyzed immediately after weighing back, along with composite samples of the feeds fed during each period.

Daily milk weights were recorded for each animal. Milk samples were taken for four consecutive milkings each week and composited for butterfat determinations every 2 weeks. Body weights were recorded for each animal at the beginning of the experiment, every 4 weeks during the period, and at the conclusion. The animals were weighed for three consecutive days, and the average of the three weights was taken as the animals' weight.

RESULTS AND DISCUSSION

Dry matter intake and digestibility. The intake of dry matter by the cows as furnished by grain, hay, silage, and pasture is shown in Table 1. Examination of the data shows that Group II consumed an average of 8.8 lb. of dry matter per cow daily from the alfalfa hay. This varied from 5.6 to 15.6 lb. daily. Group III, which received the silage as a supplement, ate an average of 4.1 lb. of dry matter per cow daily from this source. The variation for this group was 1.4 to 12.7 lb. per cow daily. The amount of dry matter furnished by the grain mixture was almost identical for the three groups. The total dry matter intakes were 31.2, 30.7, and 31.8 lb. per cow daily for Groups I, II, and III, respectively. Statistical

TABLE 1

Total dry matter intake by cows with and without supplementation

	Group										
	I			II				III			
	Control			Hay as supplement				Silage as supplement			
	Intake from:			Intake from:				Intake from:			
Period	Grain	Past.	Total	Grain	Hay	Past.	Total	Grain	Silage	Past.	Total
	(lb.)										
6/4–6/8	4.4	26.4	30.8	4.5	12.0	12.8	29.3	4.4	4.4	15.8	24.6
6/18–6/22	4.4	20.4	25.3	4.5	5.8	23.2	33.6	4.4	1.4	23.4	29.2
7/2–7/6	4.0	28.5	32.5	4.0	5.6	17.9	27.5	4.0	2.6	26.8	33.5
7/16–7/20	4.0	25.9	30.0	4.0	6.6	20.0	30.6	4.4	1.6	27.9	33.9
7/30–8/3	3.5	31.7	35.2	3.5	7.6	21.5	32.6	3.7	2.2	30.4	36.3
8/13–8/17	3.5	28.8	32.3	3.5	8.8	16.2	28.5	3.7	3.4	25.7	32.8
8/27–8/31	3.2	27.4	30.6	3.2	8.8	19.6	30.8	3.4	4.2	24.2	31.9
9/10–9/14	3.2	30.0	33.2	3.2	15.6	13.6	32.4	3.4	12.7	16.6	32.6
Average	3.8	27.4	31.2	3.8	8.8	18.1	30.7	3.9	4.1	23.8	31.8
Percentage change from control						−33.9%	− 1.6%			−13.1%	1.9%

analysis showed that there were no significant differences between groups for total dry matter intake. However, there was a highly significant difference between Groups I and II in dry matter intake from pasture, with the difference between Groups I and III approaching significance at the 5% level of probability. Thus, it is apparent that supplementation did significantly decrease pasture consumption but had no significant effect on total dry matter intake. On a percentage basis and in comparison with the control ration, hay feeding decreased dry matter consumption from pasture by 33.9%, and silage feeding reduced it by 13.1%.

TABLE 2

Apparent dry matter digestibility of rations by cows with and without supplementation as based on the chromogen (s) technique (by periods)

	Group		
	I	II	III
Period	No supplementation	Hay as supplement	Silage as supplement
	(%)	*(%)*	*(%)*
6/4–6/8	59.2	57.8	56.9
6/18–6/22	62.8	64.4	63.6
7/2–7/6	69.3	64.3	67.6
7/16–7/20	66.3	65.6	65.7
7/30–8/3	67.9	65.9	66.8
8/13–8/17	64.5	61.4	64.8
8/27–8/31	65.0	61.8	62.2
8/10–9/14	55.4	54.8	55.4
Average	63.8	62.0	62.9

Determinations as to apparent digestibility of the entire ration (Table 2) show no consistent variation due to differences in ration. The lowest apparent digestibility was found for the eighth (and last) sampling period when the pasture forage was very dry and brown and was rated as being poor in quality. Seasonal apparent digestibility coefficients for dry matter averaged 63.9, 62.0, and 62.9% of the control group, the alfalfa hay group, and the silage group, respectively. A highly significant difference ($P < 0.01$) in ration digestibility was noted between rations fed Groups I and II. No significant differences were found between rations I and II or II and III.

Milk production. Yields of fat-corrected milk (FCM) for weekly periods are shown in Table 3. These data show very little variation between groups from the start of the experiment up until the middle of July, when there appeared to be some differences in favor of the supplemented groups. This was especially true of Group II during the last 6 weeks of the trial. All three groups produced about the same amount of fat-corrected milk at the beginning of the trial. After removing differences in original production there was no significant difference found between the production of the three groups during the 16-week trial when analyzed by analysis of covariance (*8*). Milk production during the latter part of the trial appeared to favor hay and silage supplemented groups, but the differences were too small to be significant.

TABLE 3
Average daily production of fat-corrected milk (by weeks)

Expt. week	Dates (1955)	Control group	Hay group		Silage group	
		(lb.)	*(lb.)*	*(% control)*	*(lb.)*	*(% control)*
Prelim.	5/19–5/25	35.3	35.7	101.1	35.8	101.4
1	5/26–6/1	35.8	35.1	98.0	35.0	97.8
2	6/2–6/8	35.2	33.0	99.4	32.3	97.3
3	6/9–6/15	31.1	32.3	103.9	30.0	96.5
4	6/16–6/22	30.8	31.9	103.6	30.5	99.0
5	6/23–6/29	29.7	31.7	106.7	29.3	98.7
6	6/30–7/6	28.4	27.8	97.9	29.8	104.9
7	7/7–7/13	26.6	26.4	99.2	27.0	101.5
8	7/14–7/20	25.8	27.4	106.2	26.5	102.7
9	7/21–7/27	24.9	27.0	108.4	26.6	106.8
10	7/28–8/3	25.4	25.2	99.2	24.3	95.7
11	8/4–8/10	23.5	24.9	106.0	23.7	100.9
12	8/11–8/17	23.6	26.0	110.2	23.6	100.0
13	8/18–8/24	22.9	26.1	114.0	22.1	96.5
14	8/25–8/31	20.9	25.8	123.4	20.4	97.6
15	9/1–9/7	20.3	26.1	128.6	22.1	108.9
16	9/8–9/14	17.4	22.0	126.4	21.6	124.1
16-week average		26.3	28.0	106.5	26.6	101.1

All groups averaged high in monthly (4-week) persistency of milk production with no consistent trend favoring either group, indicating that the nutrient intake for all groups was sufficient to maintain a relatively high level of milk production. This was unexpected in view of the observation that the pasture was grazed rather closely and appeared to be dry and brown, especially during the latter part of the experimental period.

Body weight changes. The total gains in body weight per cow were 1.7 lb. for Group I, − 4.8 lb. for Group II, and 11.5 lb. for Group III. These small differences in body weight changes were not statistically significant.

SUMMARY

During the summer of 1955, 18 lactating dairy cows while on pasture were used in a 16-week experiment to measure what influence the feeding of alfalfa hay or alfalfa silage as a supplement would have on milk production, body weight changes, total dry matter intake, and ration digestibility.

Estimates of dry matter intake indicated that neither supplement significantly increased total dry matter intake; however, the portion of the dry matter received from pasture was reduced 33.9% as a result of hay feeding and 13.1% as a result of silage feeding. The apparent digestibility of the ration by cows fed hay was significantly lower than that of the other two rations.

Supplementation failed to produce a significant increase in milk production although there was a tendency for the groups receiving the hay and silage to produce slightly more fat-corrected milk during the latter half of the experiment. There were no significant changes in body weights.

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STUDIES ON THE RATE OF MACHINE MILKING OF DAIRY COWS. I. NORMAL VARIATIONS [1]

W. E. STEWART, L. H. SCHULTZ, AND S. P. COKER
Cornell University, Ithaca, New York

Milking rates were obtained during different and during consecutive stages of lactation for Brown Swiss, Guernsey, Holstein, and Jersey breeds. The average milking was about 3.5 minutes and the average stripping was 37 seconds. The milking rate decreased from about 4.5 minutes in the early lactation, to about 3.4 minutes in the middle, and to about 2.8 minutes in the late, lactation. For cows managed under comparable conditions, 36% milked out in less than three minutes, 38% in three to four minutes, 17% in four to five minutes, and 9% required over five minutes. *Editor.*

In order to make recommendations to dairymen regarding milking practices, it is desirable to know what can be expected in terms of milking rate when a proper milking procedure is followed. In separate experiments, Dodd (*3*) and Beck *et al.* (*1, 2*) have conducted such studies. Both found definite variations between cows in required milking time. Since the studies of Dodd were conducted in England, with a breed of minor importance in this country, and the studies of Beck were restricted to one herd, it was felt desirable to obtain additional information with cows of different breeding. This work was reported earlier in abstract form (*10*).

EXPERIMENTAL PROCEDURE

A standard single unit, pail-type De Laval milker suspended from a spring-type scale was used throughout this study. The scale had been fitted into the frame of a small wooden stand, which could be placed between the cows at milking time. The machine was operated at 12.5 inches of vacuum and 48 pulsations per minute.

All the cows were milked at the regular afternoon milking time. This represented an 11-hour accumulation. Each udder was cleaned and massaged with an individual towel dipped in warm chlorine solution. This was immediately followed by the use of the strip cup. The machine was applied as soon as let-down had occurred. When the last teat cup was in place, the stop watch was started. Scale readings were taken at 15-second intervals until the milk flow fell to 0.3 of a pound, or less, between readings. At this point machine stripping was started and continued until the cessation of flow; whereupon, the stop watch was stopped and the machine removed.

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[1] Supported in part by the De Laval Separator Company, Poughkeepsie, New York.

All cows were milked twice (on consecutive afternoons) with the second day's record being used in the analysis. An attempt was made to milk each cow that freshened in the Cornell herd in three consecutive stages of the same lactation, and as close as possible to 50 days after calving (early lactation), 150 days (middle lactation), and 250 days (late lactation). Early lactation was designated as any milking up to 100 days after calving, middle from 101 to 200 days, and late lactation as any milking after the 200th day.

Rate of milking measurements were obtained over a four-year period on 286 different cows of the Brown Swiss, Guernsey, Holstein, and Jersey breeds. Of these, 265 were milked in early lactation, 232 in middle lactation, and 196 in late lactation.

Eight rates of milking measurements were used in studying the data: (1) machine time start to stripping, (2) machine stripping time, (3) pounds of milk from machine stripping, (4) total milking time, (5) total pounds of milk, (6) average rate of flow (total pounds of milk divided by total milking time), (7) maximum rate of flow (fastest minute), and (8) time to the start of maximum rate of flow.

RESULTS AND DISCUSSION

Over-all averages. Over-all averages for the 286 cows are included in Part I, Table 1. These cows required an average of three minutes and 33 seconds total milking time. Beck *et al.* (*2*) obtained a three-minute average total milking time for 48 cows, milked in all three stages of lactation, with a machine operated at 15 inches of vacuum and 48 pulsations per minute. It may be that this higher vacuum level was a factor in shortening the total milking time. Also, the cows in Beck's study averaged 11.5 pounds, compared to 13.5 pounds in this study.

Average machine-stripping time and yield were similar in the two studies. Cows in the Beck *et al.* (*2*) study averaged 42 seconds in stripping time and 1.6 pounds in stripping yield, and the cows in this study averaged 37 seconds in stripping time and 1.5 pounds in stripping yield. This average of 1.5 pounds of machine strippings equals what Dodd and Henriques (*5*) obtained for 100 randomly selected cows.

Whereas Dodd (*3*) observed a 9.06-minute average total milking time for 326 milkings of 141 cows at five to six weeks after calving, with a machine operated at 15 inches of vacuum and 48 pulsations per minute, the 265 cows milked in early lactation in our study required only four minutes and 24 seconds total milking time. The fact that the data on Dodd's cows were collected in the morning and ours in the afternoon does not explain this great difference in speed of milking, even though Dodd's cows produced more milk (23.57 pounds as compared to 18.6 pounds in our study). Apparently, other factors such as individual and breed variation were responsible for most of the difference.

As has been reported by Matthews *et al.* (*8, 9*), Dodd *(3)*, Dodd and Foot *(4)*, Beck *et al. (2)*, and Gregoire *et al. (6)*, this study showed that each cow had a rather characteristic rate of flow for a given production level.

TABLE 1

Rate of milking measurements

Group	No. cows	Av. days in milk	Machine time *min.–sec.*	Machine stripping time *min.–sec.*	Machine stripping *lb.*	Total time *min.–sec.*	Total *lb.*	Rate *lb/min* Av.	Rate *lb/min* Max.	Time to start of maximum rate *min.–sec.*
Part I — Over-all averages (286 cows)										
Early	265	48	3:48±1:08[b]	0:36±0:27	1.5±1.3	4:24±1:19	18.6±5.9	4.4±1.5	6.4±2.5	0:59±0:45
Middle	232	153	2:47±0:57	0:37±0:24	1.6±1.2	3:24±1:03	13.0±4.9	3.9±1.1	5.7±2.2	0:34±0:35
Late	196	260	2:11±0:50	0:38±0:27	1.4±1.0	2:49±0:57	8.8±3.6	3.2±1.1	4.4±1.9	0:23±0:29
Combined[a]		154	2:56±1:12	0:37±0:26	1.5±1.2	3:33±1:19	13.5±6.4	3.8±1.3	5.5±2.4	0:39±0:41
Part II — Effect of stage of lactation (98 cows milked in three stages of same lactation)										
Early	98	48	3:56	0:32	1.3	4:28	18.9	4.4	6.4	0:56
Middle	98	154	2:53	0:32	1.4	3:25	13.2	4.0	5.7	0:37
Late	98	255	2:09	0:40	1.4	2:49	8.4	3.0	4.1	0:25
Stage differences			**	**	non	**		**	**	**
Part III — Effect of breed (early lactation)										
B. Swiss	43	50	3:53±1:06	0:30±0:22	1.2±1.2	4:23±1:14	15.5±4.9	3.6±1.0	4.9±1.6	0:72±0:35
Guernsey	24	50	4:04±1:03	0:44±0:44	1.4±1.0	4:48±1:28	14.1±4.5	3.0±0.8	4.1±1.3	0:67±0:56
Holstein	169	47	3:51±1:12	0:38±0:26	1.7±1.3	4:29±1:21	20.8±5.4	4.9±1.5	7.2±2.5	0:59±0:46
Jersey	29	48	3:13±0:35	0:24±0:11	1.0±0.7	3:27±0:45	14.4±4.4	4.0±1.1	5.9±1.9	0:37±0:28
Breed differences			*	*	**	**	**	**	**	**
Part IV — Range (lowest to highest)										
All cows	286		0:45 to 9:30	0:00 to 2:20	0.0 to 8.3	1:15 to 11:40	3.4 to 32.0	1.4 to 9.7	1.3 to 14.0	0:00 to 4:30

[a] The combined figures represent simple unweighted averages of the three stages.

[b] Arithmetic mean and standard deviation.

* Significant at the 5% level.

** Significant at the 1% level.

Effect of stage of lactation. Ninety-eight cows were milked in the three consecutive stages of the same lactation. This group included 19 Brown Swiss, 10 Guernseys, 53 Holsteins, and 16 Jerseys. These cows averaged 2.8 lactations in milk. Total milking time (Part II, Table 1) decreased significantly as the lactation progressed. The decrease was from four minutes and 28 seconds in early lactation, to three minutes and 25 seconds in middle lactation, to two minutes and 49 seconds in late lactation. This observation is similar to the findings of Dodd (*3*), Beck *et al. (2)*, and Gregoire *et al. (6)*, but is in contrast to the observations of Whittleston *(11)*. His data indicated that total milking time remained approximately the same throughout the lactation. He attributed this to erratic delays in start of milk flow and a decreased average rate of flow. Cows in this study also had a decreased average rate of flow as the lactation progressed, but the decrease in average rate of flow was not as rapid as was the decrease in production. Thus, reduced total milking times were obtained. Matthews *et al.* (*9*), Dodd *(3)*, Harshbarger *(7)*, and Beck (*1*) also reported a decrease in average rate with advancing lactation; whereas, Gregoire *et al. (6)* observed a tendency for it to increase.

Another factor which may have been important in the decrease in total milking time obtained in this study, was that machine-stripping time and yield remained approximately the same throughout the lactation. A similar phenomenon has been reported by Whittleston *(11)* and Beck *(2)*. The other important factor, the time to the start of maximum rate of flow, decreased significantly with advanced lactation in this study, which is in agreement with Beck *et al. (2)* and Gregoire *et al. (6)*, but contrary to Whittleston's *(11)* findings.

Effect of breed. Many workers have suggested that speed of milking is an inherited characteristic, but not enough information is available to determine its heritability. The breed data (Part III, Table 1) from our study also suggest the inheritance of speed of milking. There was a significant or highly significant difference between breeds in all of the milking measurements studied. Beck's data (2) on breed differences, and the data in this study, are quite similar. In both studies the Jerseys had shortest total milking time, the Holsteins the fastest average and maximum rates of flow, and the Guernseys the lowest rate of flow and the longest total milking time.

It should be recognized that these results on breed differences apply only to the Cornell herd and not necessarily to the breeds as a whole. The numbers in certain breeds were admittedly very small. The data do suggest, however, that rate of milking is an inherited characteristic.

Variation between cows. Great variation in rate of milking was observed between cows (Part IV, of Table 1). Total milking time in all stages of lactation varied from one minute and 15 seconds to 11 minutes and 40 seconds, and maximum rate of flow from 1.3 to 14.0 pounds per minute. The between-cow variation was also found by Dodd *(3)* to be much greater than the within-cow variation. He found that the total milking time varied from 4.42 to 21.50 minutes for the 326 milkings made in early lactation (fifth to sixth week after calving). Of these,

only 2.8% required less than five minutes, and 8.13% required over 14 minutes. Beck et al. *(2)* found that the total milking time in early lactation averaged 3.5 minutes with a range of from two to seven minutes. Data in this study show a range in total milking time in early lactation of from two minutes and 35 seconds to 11 minutes and 40 seconds with a four-minute-24-second average.

In the data of Table 2, cows have been grouped according to their total milking times. For the 232 cows milked in mid-lactation, averaging 153 days after calving,

TABLE 2

Variation between cows: Percentage of cows that milked out in the various time intervals

Breed	Stage of lact.	No. cows	Less than 3 min.	3 to 4 min.	4 to 5 min.	Over 5 min.
			(%)			
Brown Swiss	E	43	5	30	37	28
	M	35	31	43	17	9
	L	33	64	18	15	3
Guernsey	E	24	4	29	25	42
	M	20	35	40	15	10
	L	17	47	35	18	—
Holstein	E	169	8	27	34	30
	M	152	33	38	20	10
	L	122	57	34	5	3
Jersey	E	29	21	38	38	3
	M	25	60	32	4	4
	L	24	79	17	4	—
All cows	E	265	9	29	34	28
	M	232	36	38	17	9
	L	196	60	29	8	3
Combined (693 milkings)			32	32	21	15

it was found that 36% required less than three minutes, 38% from three to four minutes, 17% from four to five minutes, and 9% over five minutes. Beck *et al. (2)* found that 76% of the cows milked out in four minutes or less in early lactation; whereas, in this study, 38% milked out in less than four minutes.

SUMMARY

Rate of milking measurements were obtained on 286 different cows of the Brown Swiss, Guernsey, Holstein, and Jersey breeds over a four-year period. Of these, 265 were milked in early lactation, 232 in middle lactation, and 196 in late lactation. For the 286 cows, averaging 2.8 lactations and 154 days in milk, the total milking time was three minutes and 33 seconds. The average machine-stripping time was 37 seconds, which resulted in 1.5 pounds of strippings.

Ninety-eight cows were milked in the three consecutive stages of the same lactation. Both the rate of milking and total milking time decreased significantly with advanced lactation. Total milking time decreased from four minutes and 28 seconds in early lactation, to three minutes and 25 seconds in middle lactation, to two minutes and 49 seconds in late lactation. However, the machine-stripping time and yield remained approximately the same from early to late lactation.

Forty-three Brown Swiss, 24 Guernsey, 169 Holstein, and 29 Jersey cows were milked in early lactation. Statistically significant variation was found between these breeds in rate of milking, suggesting that rate of milking is an inherited characteristic. The Jerseys required the shortest total milking time, three minutes and 37 seconds, and the Holsteins had the fastest average and maximum rates of flow, 4.9 and 7.2 pounds per minute, respectively.

Great variation was found between cows in milking rate. Total milking time varied from one minute and 15 seconds to 11 minutes and 40 seconds, and maximum rate of flow from 1.3 to 14.0 pounds per minute. Two hundred and thirty-two cows were milked in mid-lactation (153 days in milk), with 36% milking out in less than three minutes, 38% in from three to four minutes, 17% in from four to five minutes, and 9% taking over five minutes.

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GRAIN-EQUIVALENT OF IMMATURE ALFALFA FOR MILK PRODUCTION WHEN FED AS SOILAGE AND AS SILAGE [1]

C. F. HUFFMAN, S. T. DEXTER, C. W. DUNCAN, AND C. A. LASSITER
Departments of Dairy, Farm Crops, and Agricultural Chemistry
Michigan State University, East Lansing

This investigation reports the effects on milk production of replacing either all or part of the grain or hay with immature alfalfa soilage and silage. Replacing either silage or soilage for part of the hay in an all-hay ration increased daily FCM, and replacing all of the hay with silage increased daily FCM on less TDN than the cows received on the hay ration. The silage and the soilage were equal in grain-equivalent for milk production on a dry-matter basis. *Editor.*

The relative grain-equivalent value for milk production of immature alfalfa fed as soilage and as silage has not been studied extensively. With the exception of pasture, soilage was probably the first method of feeding roughages to cattle. It was called soilage because freshly harvested roughage was placed on the ground for livestock to eat. The early literature on soilage was reviewed by Kildee *et al. (4)*. Prior to the development of adequate machinery, the soilage system of livestock feeding required too much hand-labor. Development of farm machinery for harvesting and hauling freshly cut crops to the feed lot has resulted in a revival of interest in soilage, or zero-grazing, as it is sometimes called. Increased use of hay-crop silage has also resulted from the development of farm machinery which has reduced the labor in making silage. The first attempt to compare the relative value of crops fed as soilage and as silage was reported by Graves *et al. (1)*.

The purpose of this investigation was to study the effect on milk production (a) when either all or part of the grain or hay was replaced with immature alfalfa fed as soilage, and (b) when silage replaced either all or part of the grain or hay.

EXPERIMENTAL PROCEDURE

A good stand of alfalfa was used for this study. On May 15, when the alfalfa was about one foot tall, a batch was harvested daily and fed in the barn for a period of 15 days. At the end of this period, the alfalfa was from 18 to 24 inches in height and contained about 82% moisture. Moisture determinations were made daily. A strip of alfalfa adjoining that which was being used for soilage was mowed on May 23 for silage. Due to unfavorable wilting weather, the al-

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falfa was not ensiled until May 25, and then 100 lb. of dry molasses per ton were added as a preservative, because the moisture content was still too high. Quality of the silage was excellent. The pH was 4.4.

Nineteen Holstein cows were used in 29 trials. The cows received the various basal rations from 18 to 75 days prior to the replacement with fresh prebud alfalfa or prebud alfalfa silage. Milk production for the 12-day basal period prior to feeding soilage or silage was used to measure the decline in milk associated with advance in lactation. The experimental period following the basal period was either 15 or 18 days in length. At the end of the experimental period, the cows were changed back to the original basal ration for 15 days, except for a few nine-day periods. Daily milk production for the two basal periods was averaged and compared with the amount of milk produced during the experimental period. Milk production is expressed as fat-corrected milk (FCM). Grain supplement consisted of either corn or corn and soybean oil meal. The cows were weighed once each three-day period.

Previous work *(2)* indicated that the response to the grain factor usually reached a maximum on the fifth to the seventh day after part of the hay was replaced with grain, and a decrease in milk production occurred just as rapidly when the grain was replaced with hay.

Composition of the experimental feeds and the coefficients of digestibility are presented (Table 1). Dry matter content of the freshly cut alfalfa averaged about 17.2% for the 15-day feeding period. Coefficients of digestibility used for soilage were those reported by Woodman *et al. (9)* for prebud alfalfa (10 to 18 inches in height at the start of the digestion trial and 17 to 24 inches at the end). Morrison's *(6)* coefficients of digestibility were used for feeds No. 8, 9, and 10. No data are available in the literature for early-bud brome hay (No. 4); therefore, Schneider's *(7)* coefficients for immature timothy hay were substituted for the brome. Total digestible nutrient (TDN) content of the dried cane molasses was estimated. The high ash and fiber content indicates that a mineral compound and straw (rice) had been added to keep the molasses dry. These data together with the amount of nitrogen-free extract were used as a basis for estimating the TDN value. Coefficients of digestibility were determined for feeds No. 2, 3, 5, 6, and 7 (Table 1).

RESULTS

The immature fresh alfalfa had the following percentage composition on the dry basis: ash, 9.5; protein, 23.4; ether extract, 3.3; crude fiber, 24.5, and NFE, 39.2, compared to Woodman *et al. (9)* values of 12.1, 25.3, 2.3, 22.1, and 38.1, respectively. Alfalfa used in this study was slightly higher in ether extract, crude fiber, and NFE, but lower in ash and protein than that used by Woodman *et al.* The silage made from immature alfalfa had the following percentage composition on the dry basis: ash, 9.9; protein, 21.7; ether extract, 4.6; crude fiber, 27.1, and NFE, 36.7. This silage was somewhat higher in ash, ether extract, and crude fiber than the alfalfa fed in this study or that used by Woodman

TABLE 1

Percentage composition and digestion coefficients of the experimental feeds

Feed No.	Description	D.M.	Ash	Protein	Ether extract	Crude fiber	NFE	TDN
1	Alfalfa, prebud[a] (cut daily)	17.2	1.64	4.0 84[b]	0.57 38	4.2 64	6.8 80	12.0
2	Alfalfa silage, prebud	38.0	3.78	8.3 70.1	1.75 70.5	10.3 55.4	13.9 65.9	21.9[e]
3	Clover-timothy, full bloom (U.S. No. 2, first cutting)	88.9	5.55	9.6 56.2	2.02 34.7	28.6 53.7	43.1 66.3	50.9
4	Brome, early bud (U.S. No. 2, extra-leafy)	89.8	6.25	11.0 54[c]	2.31 34	28.5 75	41.7 65	56.2
5	Alfalfa — 20% clover (U.S. No. 3, Alfalfa 0.5 bloom)	89.6	5.85	13.6 55.5	1.84 36.8	30.0 53.1	38.3 58.1	47.3
6	Alfalfa-clover-quack (First cutting, 0.1-0.5 bloom)	88.9	5.61	10.9 65.2	1.50 22.7	32.1 54.2	38.8 63.9	50.1
7	Alfalfa, 0.5-0.75 bloom (Color, U.S. No. 3; leaf, No. 2)	89.0	6.04	15.4 68.5	1.32 25.2	29.9 53.1	36.3 66.8	51.4
8	Alfalfa, 0.1 bloom (U.S. No. 1 on color and leaf)	88.7	5.50	16.7 73[d]	1.64 28	25.7 46	39.2 72	53.3
9	Corn (U.S. No. 2)	87.4	3.03	9.1 77[d]	3.91 90	2.9 57	68.5 93	80.3
10	Soybean oil meal (Solvent process)	89.6	5.34	47.2 92[d]	0.86 47	5.5 87	30.7 94	78.0
11	Cane molasses, dried (Silage preservative)	89.6	15.30	14.9	2.00	6.5	50.9	55.0[e]

[a] First line represents the chemical composition; second line, the digestion coefficients.
[b] Woodman *et al. (9)* values for prebud alfalfa fed as soilage.
[c] Schneider's *(7)* values.
[d] Morrison's *(6)* values.
[e] Estimated values.

et al. Coefficients of digestibility determined for protein, crude fiber, and NFE in the silage made from immature alfalfa are lower than those reported by Woodman *et al.* for prebud alfalfa, but the coefficients are in good agreement with those reported by Schneider *(7)* for very immature alfalfa hay. TDN values for soilage and silage, on the dry basis, were 69.8 and 57.6, respectively. The lower value for silage may have been due, in part, to the use of dried molasses as a preservative. The ether extract value was not multiplied by 2.25 in the calculation, because of the large amount of extractable material present in the silage that was not fat.

Results of replacing all or part of the daily grain supplement with immature alfalfa fed as soilage are presented (Table 2). In Trials 1, 2, and 3, the production of FCM declined from 0.9 to 1.8 lb. per day when the grain was replaced with soilage; however, no change occurred in Trial 4. In Trials 5 to 9, the amount of FCM increased from 1.4 to 2.5 lb. per day (av. 1.8) when 50 lb. of soilage (8.7 lb. of dry matter) replaced from 11.6 to 15.1 lb. of hay per day (av. 12.7).

TABLE 2

Grain-equivalent of immature alfalfa for milk production, when fed daily as soilage

Trial	In milk	Body wt.	FCM	Hay[a]	Supplement		Soilage intake		TDN
					Corn	SBOM	Fresh	D.M.	
(No.)	*(days)*	*(lb.)*							
				Basal ration followed with soilage					
1	72	1070	37.5	19.8	12	3	.....	.....	22.1
		1085	35.7	18.3	4	1	77.4	13.3	22.6
2	165	1205	30.2	23.9	9	1	.....	.....	20.2
		1195	29.3	22.9	....	..	75.8	13.0	20.8
3	162	1083	24.2	21.0	8	1	.....	.....	17.9
		1092	22.7	19.7	....	..	68.7	11.8	18.3
4	164	1046	20.5	19.8	6	1	.....	.....	15.7
		1057	20.4	19.9	....	..	52.0	8.9	16.4
5	69	1192	28.9	39.8	....	..	.....	.....	20.3
		1188	30.4	27.7	....	..	50.0	8.6	20.1
6	87	1177	28.0	39.5	....	..	.....	.....	20.1
		1175	30.3	27.7	....	..	50.0	8.6	20.1
7	294	1079	15.4	32.8	....	..	.....	.....	16.7
		1062	16.8	21.2	....	..	50.5	8.7	16.9
8	181	1038	15.0	32.1	....	..	.....	.....	16.3
		1038	16.4	19.4	....	..	50.5	8.7	15.9
9	156	1284	24.7	43.0[b]	....	..	.....	.....	22.8
		1274	27.2	27.9[c]	....	..	50.5	8.7	20.4

[a] Hay No. 3 fed in all trials except in first part of Trial 9.

[b] Fed 28 lb. of hay No. 7 plus 15 lb. of hay No. 4.

[c] Hay No. 7.

Results obtained when prebud alfalfa silage replaced all or part of the grain or all or part of the hay are shown (Table 3). In Trial 1, milk production declined 3.8 lb. per day when 33.7 lb. of silage replaced 10 lb. of grain and 4.2 lb. of hay. Similar results were obtained in Trial 2. In Trial 3, milk production declined 1.9 lb. per day when 63.4 lb. of silage replaced all of the grain and

TABLE 3

Grain-equivalent of silage made from immature alfalfa for milk production

Trial	In milk	Body wt.	FCM	Hay[a]	Supplement		Soilage intake		TDN
					Corn	SBOM	Wet	D.M.	
(No.)	*(days)*				*(lb.)*				
				Basal ration followed with silage					
1	93	1019	35.5	28.8	9	1	.....	.....	21.6
		1044	31.7	24.6	....	..	33.7	12.8	19.0
2	98	978	27.6	22.6	9	1	.....	.....	18.7
		988	25.7	21.3	1	..	30.3	11.5	17.5
3	132	1121	26.0	26.4	9	1	.....	.....	21.2
		1106	24.1	5.1	....	..	63.4	24.1	16.4
4	144	1018	32.4	29.3	9	1	.....	.....	22.7
		1017	30.0	.....	....	..	67.4	25.6	14.8
5	195	813	15.6	21.1	6	..	.....	.....	14.8
		833	15.2	.....	....	..	55.4	21.1	12.1
6	165	833	17.2	21.3	6	..	.....	.....	14.9
		840	16.5	.....	....	..	55.3	21.0	12.1
7	202	1170	15.2	28.9	2	..	.....	.....	16.1
		1185	15.7	1.9	....	..	57.0	21.7	13.4
8	238	1169	14.7	28.0	2	..	.....	.....	16.5
		1166	15.6	.....	....	..	67.5	25.7	14.8
9	165	807	15.3	21.1	6	..	.....	.....	14.8
		814	16.1	.....	6	..	38.7	14.7	13.3
10	135	827	17.4	21.4	6	..	.....	.....	14.9
		826	18.7	.....	6	..	40.0	15.2	13.6
11	166	1207	10.1	39.6	....	..	.....	.....	19.8
		1212	12.8	26.7	....	..	22.0	8.4	18.2
12	27	1181	31.2	31.8	....	..	.....	.....	15.9
		1214	32.7	19.0	....	..	22.0	8.4	14.3
13	243	1241	15.4	39.3	....	..	.....	.....	18.5
		1233	16.9	26.3	....	..	22.0	8.4	18.0
14	259	1229	18.6	39.5	....	..	.....	.....	19.8
		1143	21.5	26.7	....	..	22.0	8.4	18.2
15	41	1286	28.8	38.9	....	..	.....	.....	19.5
		1282	30.9	25.4	....	..	22.0	8.4	17.6
16	192	1217	20.8	39.2	....	..	.....	.....	18.5
		1200	22.2	27.6	....	..	22.0	8.4	17.9
17	208	1148	23.3	39.8	....	..	.....	.....	18.8
		1141	26.0	27.7	....	..	22.0	8.4	17.9
18	191	1156	14.2	35.1	....	..	.....	.....	16.6
		1134	16.1	23.4	....	..	22.0	8.4	15.9
19	77	1249	27.0	37.9	....	..	.....	.....	20.2
		1246	32.9	.....	....	..	83.0	31.5	18.2
20	292	1085	20.1	37.9	....	..	.....	.....	20.2
		1095	25.3	.....	....	..	85.0	32.3	18.6

[a] Hay No. 5 fed in Trials 1, 2, 5, 6, 9, 10, 16, 17, 18; hay No. 6 in Trials 3, 4, 7, 11, 12, 13, 14, 15; and hay No. 8 in Trials 8, 19, 20.

nearly all of the hay. Appetite for silage was a limiting factor with this cow. As a result, the TDN intake declined 4.8 lb. per day.

Similar results were obtained in Trials 4, 5, and 6, but in all six trials the TDN intake was reduced from 0.8 to 7.3 lb. per day (av. 3.2) when the silage was fed. When silage replaced all of the hay and grain (Trials 7 and 8) and all of the hay, but the grain intake was kept constant (Trials 9 and 10), a slight increase occurred in milk production on a lower TDN intake.

In Trials 11 to 18, inclusive, 22 lb. of silage containing 8.4 lb. of dry matter replaced from 11.6 to 13.5 lb. of hay (av. 12.6) in the all-hay ration. There was

an increase of 1.4 to 2.9 lb. of FCM (av. 2.1) during the silage feeding periods when the cows were consuming 1.3 lb. (av.) less TDN per day than on the all-hay ration. In Trials 19 and 20, the production of FCM increased more than five lb. per day on less TDN intake when all of the hay was replaced with silage. Hay used in Trials 19 and 20 was very good quality of U. S. No. 1, second-cutting alfalfa; nevertheless, the amount of FCM increased markedly when the prebud alfalfa silage replaced all hay in the ration.

DISCUSSION

An indirect comparison was made of the grain-equivalent of immature alfalfa when fed as soilage and as silage for milk production. When 10 lb. of grain were replaced with either soilage or silage, decline in milk production was of about the same magnitude. Cows fed silage consumed less TDN than cows fed soilage, owing to the difference in the digestibility of the two feeds and also to poorer appetites for silage. Replacement of all of the hay and grain with silage resulted in only a slight decline in FCM in three of five trials, which indicated that the grain-equivalent of the silage was almost sufficient to meet the needs of the cows. These results were to be expected, because hay cut at the usual stage of maturity has been shown to be deficient in the grain factor *(2)*. Immature hays contain more of the grain factor than mature hays.

Effectiveness of the grain-equivalent in both soilage and silage was indicated by an increase in milk production when either of these feeds replaced part of the hay in the all-hay rations. Increase in the amount of FCM was about the same for both soilage and silage, even though different cows and basal hays were used. On the dry-matter basis, the grain-equivalent of the immature alfalfa preserved as silage also was about equal to that of the soilage. These results are in agreement with those of Graves *et al. (1)*, who observed an increase in milk production, over that produced by mature hay, when grass clippings were fed as soilage. They stressed the importance of using the soilage crop at an immature stage, but they also recognized the difficulty of supplying cows with good immature roughage either as pasture or soilage, owing to the decrease with maturity in the nutritive value of most roughages. These investigators also stated that immature grass silage was more stimulating to milk flow than immature hay.

Loss of nutrients in silage-making has been reviewed by Shepherd *et al. (8)*, but the results of this investigation indicate that the grain-equivalent in immature alfalfa was conserved when this crop was made into silage.

SUMMARY

Feeding immature alfalfa as soilage for 15 days as a grain replacement resulted in a decline in FCM in four trials. Replacing 11.8 to 15.8 lb. of hay in an all-hay ration with 8.7 lb. of soilage dry matter resulted in an increase of 1.4 to 2.5 lb. of FCM per day in five trials.

Good quality silage was made from a strip adjoining the immature alfalfa fed as soilage. When this silage replaced various amounts of grain, FCM declined.

When 8.4 lb. of dry matter in silage replaced from 11.6 to 21.0 lb. of hay in an all-hay ration in eight trials, FCM increased from 1.4 to 2.9 lb. per day per cow. In two trials, the replacement of all of the hay with silage resulted in an increase of 5.9 and 5.2 lb. of FCM per day on less TDN than the cows received on the hay ration. Calculated TDN intakes from silage were less than those from soilage.

Results indicate that immature alfalfa fed as soilage or silage was approximately equal in grain-equivalent for milk production, on the dry-matter basis.

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APPLICATION OF GAS-LIQUID PARTITION CHROMATOGRAPHY TO THE STUDY OF VOLATILE FLAVOR COMPOUNDS

W. G. JENNINGS

Department of Dairy Industry, University of California, Davis

Small amounts of compounds of bacterial and of chemical origin are not usually detected in milk products by ordinary chemical methods. Gas-liquid partition chromatography will, in some cases, remove this quantitative barrier and make it possible to more easily classify off-flavors. *Editor.*

Until recently, the separation of small quantities of volatile mixtures was frequently difficult and sometimes not possible. Unless chemical means of separation could be used, micro-fractionating devices were required. These demand certain minimal quantities, and if the boiling points of the volatiles are not sufficiently diverse, considerable efficiency is lost.

About 15 years ago, Turner *(15)* suggested the principles of gas chromatography. Martin and Synge *(10)* extended these principles to volatiles and suggested the process of gas-liquid partition chromatography. Various workers *(1-4, 12, 14, 15)* have applied these techniques to hydrocarbon analyses, and others *(5-9, 11, 12)* have investigated such diverse materials as fatty acids, amines, alcohols, aldehydes, and ketones. The method shows promise for the study of natural flavor compounds because of the small amounts of material required and the high degree of resolution achieved.

Work reported below illustrates the application of the method to a study of volatile compounds present in commercial starter distillate. The selection of starter distillate as a starting material was influenced both by its availability and by the desirability of establishing a correlation between specific chemical compounds and flavor characteristics in cultured dairy products.

METHODS AND PROCEDURE

Gas-liquid partition chromatography apparatus. Although the theoretical treatment of the gas chromatography apparatus is somewhat involved *(7, 8, 15)*, the governing principles can be simplified. In the simplest sense, the apparatus consists of a long tube filled with inert granular material coated with a non-volatile immobile liquid. The temperature of the column is adjusted so that the materials to be separated possess suitable vapor pressures. If a mixture of volatile materials is placed on one end of the column and the tube is swept with an inert carrier gas, the rate of a particular component through the column will be, in part, a function of its relative vapor pressure. As a component works its way through the column, passing from one oil-covered granule to the next, the number of times that it dissolves, evaporates, and redissolves is tremendous.

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The resolution obtained can correspond to efficiencies of 1,000-2,000 theoretical plates *(7, 8)*. Other factors, of course, such as solubility in or association with the immobile liquid, also affect the rate of a component through the column.

The apparatus now in use in this laboratory is illustrated in Figure 1. The column consists of 11.0 ft. of 0.25-in. o.d. copper tubing containing the immobile liquid supported on size graded Celite 545. Helium (commercial grade—99%) is used as the carrier gas. A thermal conductivity cell serves as the detection unit. This is merely a Wheatstone Bridge, two legs of which are 20 Ω platinum filaments. The two ends of a 200 Ω wire wound potentiometer comprise the other two legs of the bridge. One of the platinum filaments serves as a reference, and the other is inserted in the departing gas stream. The filaments are heated electrically by means of a storage battery (0-4 volts through a rheostat). Helium has a high thermal conductivity, and when pure helium is bathing and cooling the sensing filament, its resistance is maximal and constant. The recording potentiometer is adjusted to trace its zero or base line. When a component of the mixture emerges from the column, it displaces some helium, the filament warms up, its resistance drops, and the potentiometer records the electrical imbalance.

Dimbat *et al. (1)* have discussed the influence of temperature on peak height and retention volume. A fluid-bath apparatus offers distinct advantages in that the column can be brought to the desired temperature in a relatively short period of time, and the apparatus has sufficient "thermal buffering capacity" so that bridge imbalances caused by injection of sample, air currents, etc., are

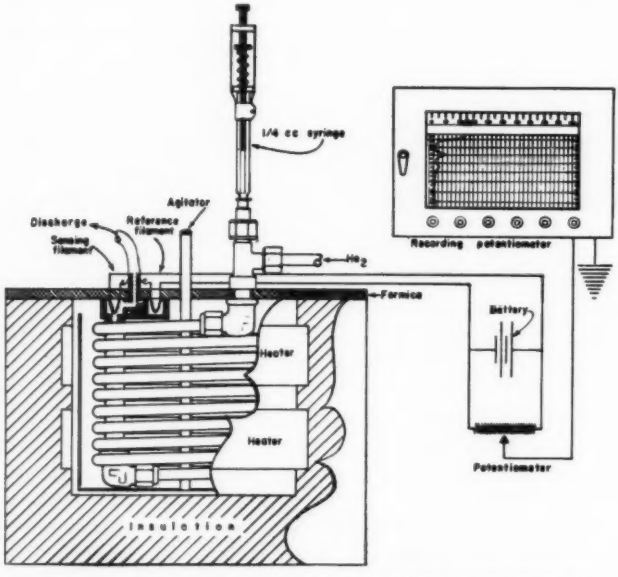

FIG. 1. Apparatus used for gas-liquid partition chromatography.

minimized. Automotive motor oil has been found satisfactory for temperatures to approximately 150° C. The apparatus illustrated uses a silicone oil for the bath fluid, and has been used as high as 220° C. Operational difficulties are not anticipated at temperatures up to 300° C. Heat is applied by two 500-w. strip heaters operated through a variable transformer. For the runs reported below, the apparatus was operated at 125°-130° C. and at a flow rate of 20 cc. per minute. One hundred ten volts was applied to the heaters until the bath temperature reached 105° C. The voltage was reduced to 30, the helium turned on, and the apparatus given 20-30 minutes to equilibrate. Under these conditions, the change in temperature with respect to time is slight and, as a run rarely exceeds 12-15 minutes, temperature can be considered constant without introducing appreciable error.

Preparation of starter distillate. In general, dilute solutions are not satisfactory for gas-liquid partition chromatography. The column on this apparatus is 4.9 mm. i.d. Liquid samples much larger than 20-25 μl do not give clean separations. The apparatus is not satisfactory for extremely dilute components; there simply is not enough of an extremely dilute component in such a small sample to activate the thermal conductivity cell.

In this work, 1 gal. of starter distillate[1] was concentrated in a modified Oldershaw column to ca. 100 cc. This material was saturated with NaCl and extracted with ethyl ether (A.R.). The major part of the ether was removed by fractional distillation, yielding ca. 8 cc. of a yellow liquid with a slight greenish tinge and a strong odor of biacetyl.

Calibration of apparatus. In order to collect individual fractions, it is necessary to calibrate the apparatus and determine the hold-up time, i.e., the time required for the vaporized component to traverse the distance from the sensing filament to the discharge tip. It is desirable to calibrate the instrument under the conditions of actual operation. This can be accomplished by the use of a material that can be conveniently detected at very minute concentration. Benzene has a high extinction coefficient and can be readily detected by ultraviolet spectrophotometry. Vaporized components may be recovered by bubbling the carrier gas through solvent, but the flow rate through the apparatus will vary with the liquid heat at the discharge tip. Consequently, accurate calibration demands a series of separate injections. A small amount (ca. 1 μl) of benzene was injected, and a stop watch was started when the recording potentiometer first responded. The gas emanating from the discharge tip for the duration of, e.g., the third second was bubbled through double distilled water. A second injection was made, and the gas discharged through the duration of the fourth second was trapped in a second tube. After a series of tubes had been prepared, the absorbancies at 255 mμ were determined. Such procedure indicated that the hold-up time for this apparatus was 9 seconds.

Preparation of the column. A number of nonvolatile liquids were investigated. These included silicone oil,[2] paraffin, glycerine, stearic acid, dinonyl

[1] Manufactured by Chr. Hansen's Laboratories, Inc., Milwaukee, Wis.

[2] Dow Corning 550 fluid, marketed by Dow Corning Corporation, Midland, Mich.

phthalate, and various combinations of these. None resolved the mixture into more than four fractions. Use of a polyoxyethylene polypropylene glycol ether[3] resolved the injected sample into six fractions.

Celite 545[4] was size graded and the 40-60 mesh fraction was isolated. The resolution obtained with this coarse particle was far superior to that obtained with ungraded Celite. The objections of Dimbat *et al. (1)* to the use of Celite apply to the use of an ungraded Celite 545. The 40-60 mesh fraction was heated at 300° C., washed with concentrated HCl, washed free of acid, and dried at 150° C.

One part of polyoxyethylene polypropylene glycol ether was dissolved in acetone, and four parts of 40-60 Celite were added. The material was slurried, and the acetone was removed on a steam bath. A piece of glass wool was inserted in one end of an 11.0-ft. piece of 0.25-in. o.d. copper tubing, and the tubing was attached to a vacuum pump. The tube was vibrated vigorously with a vibrator-type engraving tool and the coated Celite was fed into the open end. When the tube was apparently full, 30 p.s.i. He_2 was applied to one end while vacuum was maintained at the other and the tube was vibrated vigorously. The He_2 was turned off and the pressure was allowed to dissipate through the column. Unless voids had occurred during the packing, additional compaction was found to be insignificant. The column was coiled and attached to the apparatus with Swagelok[5] fittings.

Formation of derivatives. Individual fractions were condensed in various solvents by bubbling the carrier gas through solvent-containing tubes, making allowance for the hold-up time. The concentrations were increased by using the same series of tubes to collect their respective fractions from a series of injections. These solutions of isolated fractions were subjected to standard chemical procedures *(13)* to prepare derivatives.

RESULTS AND DISCUSSION

Injection of ca. 10 μl of concentrated starter distillate extract resulted in the chromatogram illustrated in Figure 2. Pip No. 1 is an air pip; a small amount of air was usually injected with the sample to serve as a possible reference point. In the ideal case, each fraction would represent a single component. Certainly some ethyl ether remained in the extract; the retention volume (the amount of gas required to carry a particular component through the column) of No. 2 agrees with that obtained for ethyl ether.

The individual fractions were collected in anhydrous pyridine and the samples were refluxed with 3,5-dinitrobenzoylchloride. Two or three crystals of a brownish solid melting at 87°-89° C. were obtained from fraction No. 4. There was not sufficient material to permit recrystallization. The ethyl ester of 3,5-

[3] Marketed as Pluronic F-68 by Wyandotte Chemical Co., Wyandotte, Mich.

[4] A diatomaceous earth marketed by Johns-Manville Corporation, New York, N. Y.

[5] A torque-free leak-proof fitting manufactured by the Crawford Fitting Company, Cleveland, Ohio.

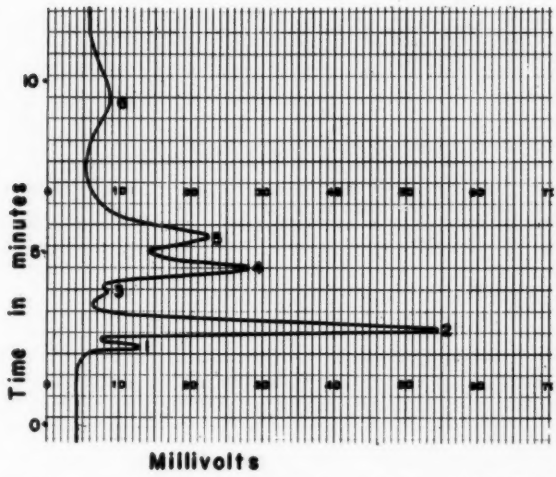

FIG. 2. Chromatogram of concentrated ether extract of starter distillate concentrate.

dinitrobenzoic acid melts at 90° C. Injection of concentrated extract containing added ethyl alcohol resulted in increasing the relative size of pip No. 4.

Individual fractions were collected in aqueous ethyl alcohol and the 2,4-dinitrophenylhydrazones attempted. No. 5 yielded an orange red material melting with decomposition at 319° C. This fraction has a strong odor of biacetyl, whose dinitrophenylhydrazone reportedly melts at 323° C.

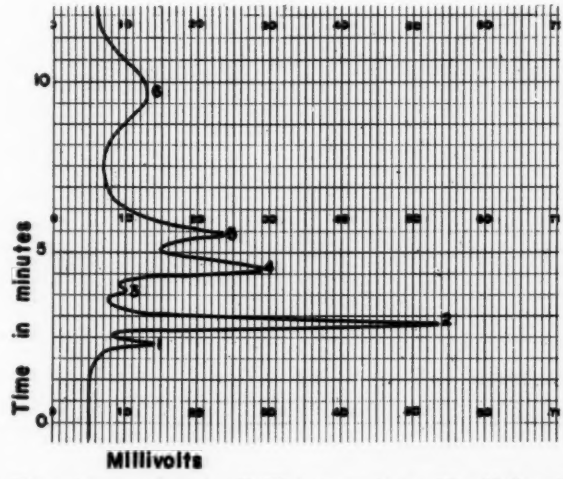

FIG. 3. Chromatogram of starter distillate concentrate with added acetic acid.

When individual fractions were collected in water and the p-phenylphenacyl esters were attempted, No. 6 gave a solid melting at 109.5°-110° C. This material was chromatographed on a column of silicic acid without changing its melting point. The acetic acid ester melts at 110.5° C. The addition of acetic acid to the concentrated extract increased the relative size of pip No. 6 (Figure 3).

Approximately 1 ml. of the concentrated extract was placed in a 25-ml. round bottomed flask; 5 ml. H_2O and a pellet of KOH were added. The material was refluxed for 4 hours. The hydrolysate was extracted five times with 10-ml. aliquots of ethyl ether, acidified with HCl and re-extracted three times with 10-15-ml. aliquots of ethyl ether. The major part of the ether was stripped from the combined extracts by fractional distillation and the material was injected. Pip No. 3 disappeared without showing hydrolysis products. If it had been ethyl acetate, the slight increase in No. 4 and No. 6 would have been difficult to detect. However, ethyl acetate mixed with the original concentrated extract does not amplify No. 3 but comes through as a separate and distinct fraction. The addition of methyl acetate, on the other hand, does increase the relative height of pip No. 3 (Figure 4). If No. 3 were methyl acetate, the hydrolysate should contain methyl alcohol. The ethyl ether used in the extraction of the hydrolysate was carefully redistilled and the last portion to come over was subjected to gas-liquid partition chromatography (Figure 5). The retention volume of the small pip following the ether pip is the same as that obtained for methyl alcohol.

This suggests that this technique would be particularly convenient for the identification of volatile acids, alcohols, and esters. The retention volume of an unknown fraction can be established as coincident with that of some known

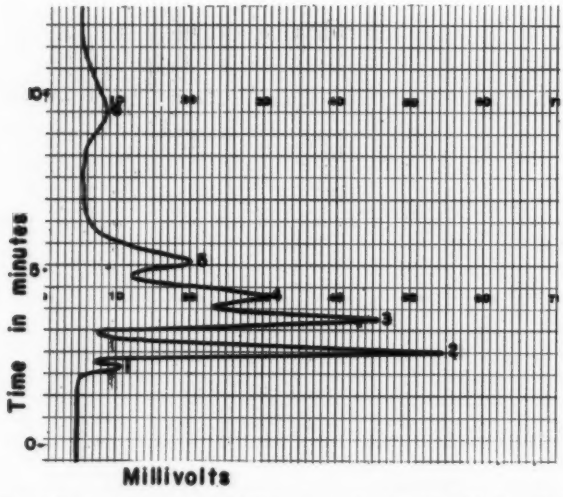

FIG. 4. Chromatogram of starter distillate concentrate with added methyl acetate.

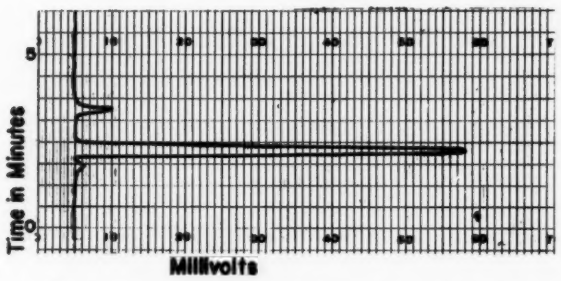

FIG. 5. Chromatogram of hydrolysate extraction ether.

compound. The retention volume of a volatile derivative (or degradation product) of the known can be compared to the retention volume of that derivative of the unknown. If these agree, it is fair evidence that the known and unknown compounds are the same. If greater certainty is desired, a similar comparison can be made with a second set of derivatives. Determination of the retention volumes of a series of compounds is not a formidable undertaking. Experimental conditions remaining constant, the logarithm of the emergence time (a function of the retention volume) is a straight line function of the molecular weight within a homologous series (Figure 6).

Certainly materials other than those identified in this study are present in starter distillate. Some, including acetoin, were excluded by confining the analyses to ether-extractable materials. Other components might require a preliminary fractionation of the starting material, so that their concentration in the injected sample would be sufficiently high to activate the detecting circuit.

SUMMARY

Gas-liquid partition chromatography is a relatively new technique holding great promise for the study of volatile compounds. The technique was applied to the ether extract of a starter distillate concentrate. Five fractions were isolated. One of these was identified as ethyl ether, which originated from the experimental procedure. The identities of three, ethyl alcohol, biacetyl, and acetic acid, were confirmed through the formation of derivatives. Methyl acetate was identified by establishing the retention volumes of the unknown and its hydrolysis products as being coincident with the retention volumes of methyl acetate and methyl alcohol and acetic acid, respectively.

Although this technique possesses many advantages, it should be recognized that it does possess certain limitations. A few of these are that the limited sample size makes it difficult to recover appreciable amounts of any given component, the sample must be reasonably concentrated and volatile, and it must be stable under the conditions employed. The method does not offer a panacea for all of the problems encountered in the study of volatiles. It can be a powerful and relatively inexpensive tool.

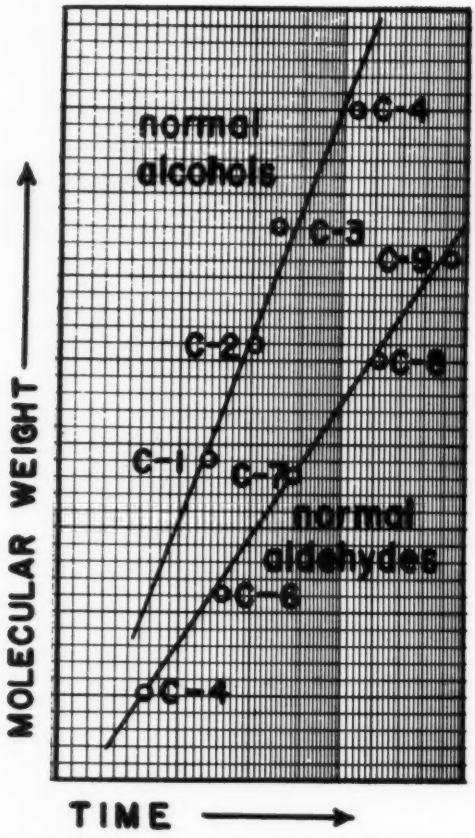

FIG. 6. Relation between emergence time and molecular weight.

ACKNOWLEDGMENTS

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EFFECT OF CERTAIN SALTS ON PRECIPITATION OF CASEIN BY CALCIUM CHLORIDE AND HEAT

C. A. ZITTLE, E. S. DELLA MONICA, AND J. H. CUSTER

Eastern Regional Research Laboratory,[1] Philadelphia, Pennsylvania

Casein was precipitated by calcium chloride and heat. It dissolved again at lower temperatures in the presence of sodium chloride and citrate but not in phosphate. Heat precipitation of casein by calcium chloride was prevented by excess citrate or phosphate and much less precipitation occurred with sodium chloride. Sodium phosphate was more effective in preventing casein precipitation at pH 6.6 than at 6.1, as more calcium phosphate was formed at the higher pH. *Editor.*

Precipitation of casein by calcium chloride and heat and the subsequent re-solution of the precipitate at lower temperature has been reported (*13*). The possible relationship of this phenomenon to the drop in viscosity of evaporated milk in storage was discussed (*13*). To relate the observations more directly to the heating of milk, the effects of sodium chloride, citrate, and phosphate on this precipitation reaction have been studied, because of the presence of these salts in milk and also because of the use of the latter two as milk stabilizers.

MATERIALS AND METHODS

Casein was prepared and the solutions were made as described previously (*13*). The pH values were adjusted after the salts or other reagents were added.

Heating of the samples, centrifuging, sampling, and determination of the casein by light absorption at 280 mμ have been described (*13*). Viscosity was determined in a Bingham type viscometer. Details of the measurement have been reported (*12*). Calcium was determined by the murexide (ammonium purpurate) method (*11*); phosphate in the concentrations used in these experiments did not interfere. Phosphorus was estimated with the ammonium molybdate reagent after reduction with ferrous sulfate.

RESULTS

Sodium chloride. The ability of increasing quantities of sodium chloride to reduce the precipitation of calcium and sodium caseinates by calcium chloride and heat is shown (Figures 1 and 1A). For the calcium caseinate experiments, calcium chloride was added to 2% calcium caseinate to give the total calcium

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[1] A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

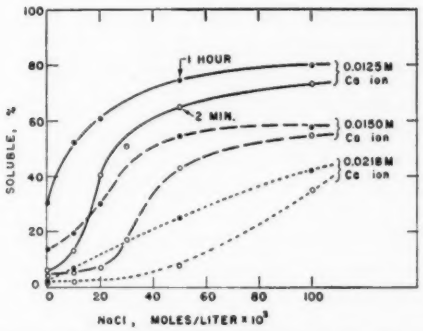

FIG. 1. Precipitation of 2% calcium caseinate heated at 90° C. for one hour in the presence of sodium chloride. The experiments were done at pH 6.5 with indicated concentrations (total) of calcium. Casein in solution at 25° C. two minutes (O O) and one hour (● ●) after heating.

concentrations indicated in Figure 1 (0.0068 *M* calcium being contributed by the calcium caseinate). For the sodium caseinate experiments, corresponding concentrations of calcium were provided wholly by the calcium chloride added, while corresponding total sodium concentrations were achieved by the sodium chloride and the 0.012 *M* sodium already present in the 2% sodium caseinate solutions. In all cases, duplicate 4.0-ml. samples at pH 6.5 were heated for one hour at 90° C., after which they were centrifuged at 25° C., one two minutes after heating and the other one hour after heating.

It is evident that sodium chloride kept the caseinates in solution; that is, it lessened the precipitation of casein when the solutions were heated. Extent of the solubilization was determined by the total sodium and total calcium and was less marked with the higher concentration of calcium. It had previously been assumed (*13*) that sodium caseinate–calcium chloride is approximately

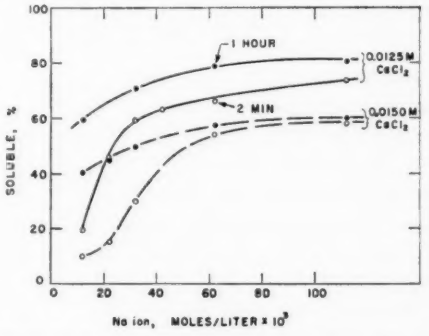

FIG. 1A. Precipitation of 2% sodium caseinate heated at 90° C. for one hour in the presence of sodium chloride. The experiments were done at pH 6.5 with indicated concentrations of calcium chloride. Curves show casein in solution at 25° C. two minutes (O O) and 1 hour (● ●) after heating.

equivalent to calcium caseinate if the effect of the sodium ion is considered. The data (Figures 1 and 1A) confirm this equivalence for calcium precipitation in the presence of sodium ion. The curves for both caseinates are almost identical for 0.0125 *M* calcium ion, and the small deviation at 0.015 *M* calcium ion is probably within the accuracy of the method. Re-solution of the precipitate at 25° C., indicated by the higher level of the "one-hour" curves as compared to the "two-minute" curves, took place, although modified quantitatively by the sodium chloride.

Sodium citrate. Effect of sodium citrate on the precipitation of 2% sodium caseinate containing 0.0125 *M* calcium chloride, pH 6.6, is shown (Figure 2). This compound was very effective at low concentrations in preventing the precipitation of casein by calcium chloride and heat. This would be expected of a compound like citrate, which has a strong binding affinity for calcium ions (*5*).

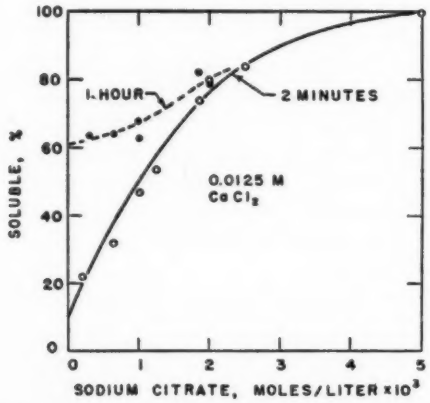

FIG. 2. Precipitation of 2% sodium caseinate containing 0.0125 *M* calcium chloride at pH 6.6 heated at 90° C. for one hour in the presence of sodium citrate. Casein in solution at 25° C. two minutes (O O) and one hour (● ●) after heating.

Sodium phosphate. Effect of sodium phosphate, (a mixture of mono- and disodium salts to give the designated pH values), on the precipitation of 2% sodium caseinate containing 0.0125 *M* calcium chloride is shown (Figure 3). Results are given for both pH 6.1 and 6.6. With sodium phosphate present, a re-solution of the casein precipitates was not obtained; hence, only one curve is shown at each pH value. The sodium chloride and citrate curves ("two minutes") are added for comparison, to show the relative effectiveness of each compound in preventing casein precipitation.

At pH 6.1 the phosphate was much less effective in preventing casein precipitation than it was at pH 6.6. At the higher pH, the solution contained additional sodium ion, added as NaOH, but that this was not responsible for the difference was shown when the same amount of sodium ion (0.0043 *M*) was added as NaCl at pH 6.1 without any change in precipitation. Other possible

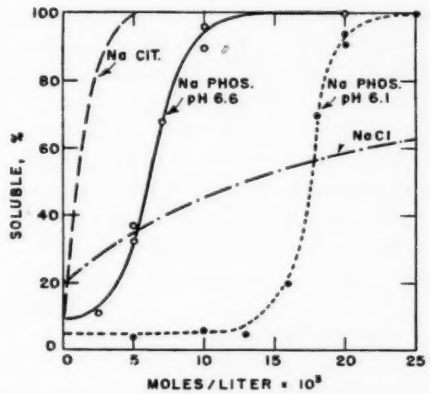

FIG. 3. Precipitation of 2% sodium caseinate containing 0.0125 *M* calcium chloride heated at 90° C. for one hour in the presence of sodium phosphate at pH 6.6 and 6.1. The "two-minute" curves for sodium chloride and citrate at pH 6.6 are also shown for comparison.

explanations are the greater tendency of casein to precipitate, or the lesser ability of phosphate to bind calcium, at the lower pH. To investigate this further, varying amounts of calcium chloride were added to sodium caseinate samples containing no phosphate and to those with a constant phosphate concentration of 0.005 *M*, and their solubilities were measured at pH 6.2, 6.6, and 7.4. As shown in Figure 4, phosphate at this concentration had no apparent effect on the precipitation of calcium caseinate at pH 6.2, but at pH 6.6 it decreased the precipitation considerably, and markedly so at pH 7.4. These results indicate that, as might be expected, phosphate has a greater affinity for calcium at the higher pH values of the limited pH range studied.

When phosphate was added to calcium-containing solutions in a concentration sufficient to prevent the precipitation of casein, the solutions were quite clear, although a small amount of sediment was apparent after centrifugation. It was of interest to know the composition of the sediment in calcium and phosphorus,

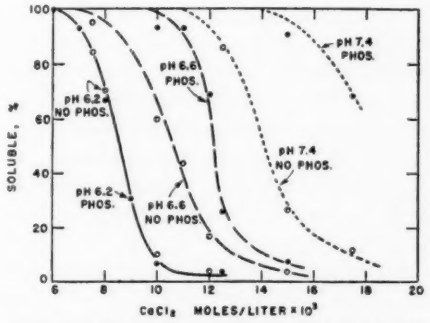

FIG. 4. Precipitation of 2% sodium caseinate with calcium chloride and heat for one hour at 90° C., without (O O) and with (● ●) 0.005 *M* sodium phosphate, at pH 6.2, 6.6, and 7.4.

and to determine whether the amount of a calcium phosphate formed was sufficient to account for the prevention of casein precipitation. In these experiments, the sample volumes were 4.0 ml., and the concentration of calcium chloride was 0.0125 *M* (calcium in 4.0 ml. = 2.00 mg.). Experiments were conducted at pH 6.1 and 6.6 with several concentrations of sodium phosphate ranging from 0.0110 *M* (P in 4.0 ml. = 1.36 mg.) to 0.0318 *M* (P = 3.94 mg.). Although at pH 6.1, and with the lower concentrations of phosphate at pH 6.6, some casein was sedimented, the casein was colloidal and could be decanted to leave the dense sediment of calcium phosphate for analysis. Results (Figure 5) are given in milligrams

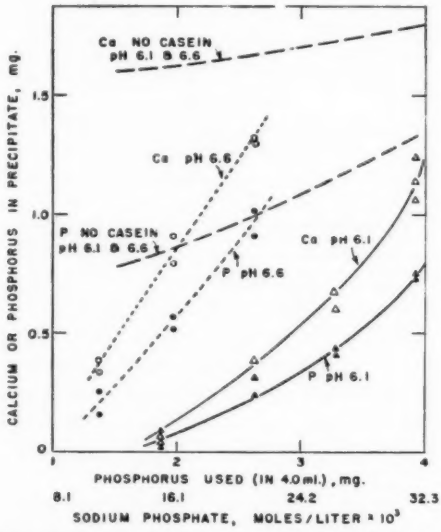

FIG. 5. Calcium and phosphorus precipitated when precipitation of casein by 0.0125 *M* calcium chloride and heat at 90° C. for one hour is prevented by excess of sodium phosphate. Also shown are curves for the precipitation of calcium phosphate (Ca and P) when no casein is present.

of Ca or P in the calcium phosphate precipitate, in relation to the milligrams of phosphate present. Each point represents a separate experiment.

Curves are also shown (Figure 5) for the precipitation of Ca and P from similar solutions when no casein is present. With these solutions the pH adjustment with NaOH was continued until the pH ceased to drop. The drop in pH on adding alkali to calcium phosphate solutions in this pH range is due to the transformation of dicalcium to tricalcium and the soluble acidic monocalcium phosphates (*10*). Even though the pH was thus carefully adjusted, the pH dropped almost one unit when the solutions were heated. This drop in pH did not occur when casein was present.

From Figure 5 can also be calculated, by subtraction from the amount originally present in the sample, the percentage of Ca and P in the supernatant fluid.

These calculations show that the formation of insoluble calcium phosphate at pH 6.6 decreased the calcium concentration 19 to 67%, depending on the phosphate concentration. It is apparent that such a formation is sufficient to account for the nonprecipitation of the casein. Other aspects of this reaction are dealt with in the discussion.

The ability of sodium phosphate to prevent the re-solution of calcium caseinate precipitates was investigated in some detail. It was found that as little as 0.00063 *M* sodium phosphate in a 2% casein solution at pH 6.6 containing 0.0125 *M* calcium chloride prevented the re-solution of the precipitate obtained by heating. Without the phosphate, when samples were heated for one hour at 90° C., the casein remaining in solution was about 12%; but after they stood for another hour at 25° C., it increased to 60% (*13*). Changes in viscosity obtained on the addition of calcium chloride to sodium caseinate, and the effect of heat on viscosity (*13*) were also explored with 0.005 *M* sodium phosphate present. In general, the results were about the same as without phosphate (*13*) up to a concentration of 0.010 *M* calcium chloride. That is, the viscosity dropped as the calcium concentration was increased and there was some decrease in the viscosity when the solutions were heated. With higher concentrations of calcium, however, the viscosity increased, presumably because a colloidal precipitate was formed. When these solutions were heated, the viscosity invariably dropped. Also, the calcium-casein precipitates appeared to be more dense when phosphate was present. In every instance, the presence of phosphate prevented the viscosity increase on heating, and the reversal at lower temperature that was obtained with sodium caseinate–calcium chloride systems (*13*). The observations were made as long as 18 hours after heating. The possibility of slow re-solution of the precipitates can not be excluded at present.

DISCUSSION

The solubilizing effect of sodium chloride on calcium caseinate precipitates is probably due to a reduction in the activity of the calcium ion, and in part to the displacement of the calcium ion by the sodium ion. Van Slyke and Bosworth (*9*) observed that acid caseinates insoluble in water were readily soluble in 5% sodium chloride. They showed by dialysis that an exchange of bases had occurred. Chanutin, Ludewig, and Masket (*3*) have reported that the affinity of calcium ion for casein in unheated solutions was decreased by the presence of sodium chloride, and Carr and Topol (*2*) have observed that sodium ions are bound to casein. It also has been reported *(4a)* that the number of micelles in skimmilk is decreased by the addition of sodium chloride, presumably by dissociation of calcium-casein complexes and formation of nonmicellar sodium caseinate.

The solubilizing effect of sodium citrate on calcium caseinate is expected, because of its strong affinity for calcium ion. According to Hastings *et al.* (*5*) the association constant, K, for the formation of calcium citrate, expressed by the ratio of the equilibrium concentrations $[Ca^{++}]$ $[Citrate^{=}]/[CaCitrate]$,

is 6.0×10^{-4} (pK of 3.22). Chanutin, Ludewig, and Masket (*3*) have investigated the influence of citrate on unheated calcium-casein solutions and found that the reduction in the amount of calcium bound to casein conformed to this association constant. On the basis of this K value, with a concentration of 0.0125 *M* total calcium and for the formation of 0.005 *M* calcium citrate, only 0.0004 *M* free citrate would be present at equilibrium. Reduction of the calcium concentration by the amount bound to citrate, together with the sodium ion effect, is sufficient to prevent the precipitation of the casein completely. Sommer and Hart (*6*) have reported qualitative tests showing the solubilizing effect of citrate on calcium citrate precipitates.

The ability of sodium phosphate to prevent the precipitation of calcium caseinate was observed by Sommer and Hart (*6*). Their qualitative studies suggested that the results were independent of heating. In the present studies, the extent of precipitation was greatly increased by heating, and all the results reported are for heated casein solutions. The magnitude of the effect of heat is shown by specific examples; solutions used for the data in Figure 4 containing 15 and 12.5×10^{-3} *M* calcium chloride per liter, together with phosphate, at pH 6.6, were completely soluble before heating. After heating to 90° C. for one hour the solubility had decreased to 7% and 26%, respectively.

It was of interest to know whether excess phosphate prevented the precipitation of casein by calcium by forming a soluble complex with the calcium caseinate, or whether the solubilization occurred because of the removal of calcium by formation of insoluble calcium phosphate. It is apparent from the data that the latter provides the explanation. The increased effectiveness of sodium phosphate at higher pH values in solubilizing calcium-caseinate precipitates lies in the formation of additional insoluble calcium phosphate; however, the reason more is formed at pH 6.6 than at pH 6.1 is not apparent. It was surmised that this difference reflected the greater affinity of calcium and phosphate at higher pH values, as shown by the greater tendency to form insoluble calcium phosphates. Experiments with calcium and phosphate without casein present indicate that this is probably not the explanation, with the small difference in pH involved. Also, the greater formation of a calcium phosphate at pH 6.6 can not be governed by the binding of calcium to the casein, for this binding is less at lower pH values (*1, 3*).

Composition of the calcium phosphate sediment is of interest; at pH 6.6 the molar ratio of Ca:P in the sediment from the low phosphate solution is 1.46, corresponding to tricalcium phosphate, decreasing to 1.08 from the high phosphate solution, corresponding to dicalcium phosphate. Titration studies (*10*) indicate that dicalcium phosphate is unstable, having a tendency to form tricalcium phosphate and the soluble monocalcium phosphate. The excess of phosphate may, however, stabilize the dicalcium phosphate, or alternately the excess phosphate might be adsorbed to the tricalcium phosphate (*7*). The molar ratio of the Ca to PO_4 remaining in solution varies from 1.08 in the low phosphate solution to 0.3 in the high phosphate solution. This ratio probably has little relation to

complex formation with casein because of the presence of free phosphate. The influence of the phosphate concentration on the composition of the sediment, however, suggests that a variable phosphate concentration in milk might equally well cause a variation in the composition of the calcium phosphate associated with casein. This might account for the variation in the composition of the casein complex from individual milk samples *(8)*. A reduction in the Ca:P ratio in the casein complex of milk has been obtained in the presence of added phosphate *(1)*.

There appears to be general agreement *(1a)* that in the casein complex of milk the tricalcium phosphate is bound to the calcium caseinate by chemical bonds and not by physical forces such as adsorption. In the case of destabilization of this casein complex by heat, however, it has been suggested that the calcium caseinate coprecipitates or is adsorbed to the precipitate of tricalcium phosphate. Also, it has been suggested that whereas calcium caseinate is most unstable in acid solution, in the presence of tricalcium phosphate it would be more unstable at higher pH values, where there is a greater tendency of tricalcium phosphate to precipitate. The present studies, however, indicate that little or no calcium caseinate coprecipitates or is adsorbed to the calcium phosphate. It has also been found (unpublished studies) that freshly precipitated tricalcium phosphate added to calcium caseinate did not remove any significant amount of casein. From this it appears that the stability of calcium caseinate, either alone or complexed with tricalcium phosphate, will be determined principally by the available calcium concentration and the pH (*13*). In the case of the calcium-caseinate–calcium-phosphate complex an excess of calcium precipitates both the tricalcium phosphate and calcium caseinate, but the sequence has not been determined. The influence of pH on the heat stability of the casein complex will be twofold; as the pH is raised more calcium is required to precipitate casein, and at higher pH values phosphate will bind calcium more strongly. The total effect will be that the heat stability of the casein will be greatly enhanced in a system of constant composition by raising the pH.

The prevention by phosphate of the re-solution of heated casein precipitates when stored at a lower temperature makes less likely the suggestion (*13*) that this phenomenon of re-solution might explain the drop in viscosity on the storage of evaporated milk. The re-solution of the casein precipitates can not be completely excluded as an explanation for the drop in viscosity, because other components of milk might make it possible for heated colloidal casein to redissolve on storage at lower temperature. Furthermore, studies at longer time intervals might reveal that a slow re-solution does occur. The ability of phosphate to prevent re-solution of casein, compared with citrate which does not interfere, emphasizes the differences between these two substances as stabilizing agents and might provide a clue to their total stabilizing effect.

SUMMARY

The effect of sodium chloride, citrate, and phosphate on the precipitation of casein by calcium chloride and heat, and the reversal of the precipitation at lower

temperature, has been investigated. The re-solution of the casein precipitate occurs in the presence of sodium chloride and citrate but not in the presence of phosphate. With excess of citrate or phosphate, the precipitation of casein by calcium chloride and heat is prevented; with sodium chloride, a large reduction in the amount of precipitate occurs. Both citrate and phosphate act by binding calcium, a reaction that in the case of phosphate leads to the formation of insoluble calcium phosphate. Sodium phosphate is more effective in preventing casein precipitation by calcium chloride and heat at pH 6.6 than at pH 6.1, because more calcium phosphate is precipitated at the higher pH.

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THE EFFECT OF HEAT AND STORAGE ON THE STABILITY OF AUREOMYCIN IN MILK, BUFFER, AND WATER

K. M. SHAHANI

Department of Dairy Technology, The Ohio State University, Columbus

Heating inactivated aureomycin in increasing order in milk, buffer, and water. This was a first-order reaction at 145° and 160° F. in milk and buffer, but not in water at 160° F. Its activity was lost during storage, but the rate was slower in the heated than in the unheated samples. Aureomycin was not completely inactivated at 143° or at 160° F. for 30 minutes. *Editor.*

Aureomycin is widely used in mastitis therapy and finds its way, in limited quantity and for a limited time, into the milk from treated cows *(1, 2, 5)*. There is a need to determine the stability of the antibiotic in milk to ascertain, *(a)* the amount which may be ingested by the consumers of milk from treated cows, and *(b)* the concentration which may remain in the milk after processing and storaging, causing starter failures in the manufacture of cultured dairy products.

In previous reports *(9, 10)*, results were presented on the effect of heat and storage on the stability of penicillin and streptomycin in milk, and some data were presented on the effect of heat on aureomycin in milk *(9)*. Additional information has been obtained on the stability of aureomycin in raw and heated milk, phosphate buffer, and distilled water. These findings are presented in this paper.

EXPERIMENTAL PROCEDURE

To determine small concentrations of aureomycin, the disk assay method reported by Dornbush *(4)* was used, with a few modifications. The lower limit of the sensitivity of the assay method was 0.025 γ per milliliter. In the present study, only one layer of agar seeded with the test organism was used, thereby reducing the time required for preparing the plates. The method was improved by pipetting 10 ml. of seeded agar in each plate, in order to have a uniform thickness. The procedure was further standardized by pipetting 0.08 ml. of the test sample onto each ½-inch S and S assay disks, instead of dipping ¼-inch disks as described by the previous workers. Brain heart infusion agar adjusted to pH 5.5 was used as the assay medium, and *Bacillus cereus* spores as the test organism. The principle of the disk assay method has been described *(10)*.

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In the study to determine the temperature and time combinations needed to inactivate aureomycin completely, fresh raw milk obtained from the University Dairy was used. It was inoculated with freshly prepared aureomycin solution, mixed thoroughly, and dispensed into screw-capped test tubes. The tubes were placed in a wire rack and quickly heated to the desired temperature by placing the entire rack in a boiling water or oil bath. An extra tube containing an accurate thermometer was placed in the rack, to observe the rise in temperature. As soon as the milk reached the desired temperature (usually it took from 45 to 110 seconds), the entire rack was quickly transferred to a thermostatically controlled bath maintained at the temperature under study. At the end of periodic intervals, a tube was removed and immersed in an ice bath. Simultaneously controlled milk samples, known to be free from antibiotics, were similarly heated and cooled in test tubes at corresponding time intervals. Immediately, the samples were assayed by the disk and the starter inhibition method *(10)* to determine residual antibiotic activity. Test milk samples which did not develop any zone of inhibition around the disk, and showed no inhibition in acid development as compared to the control, were assumed to have lost their antibiotic activity completely.

The experimental procedure to determine the effect of heat and storage on aureomycin was the same as that used in previous studies *(10, 11)*.

RESULTS

Complete heat inactivation of aureomycin in milk. Fresh raw milk was inoculated with 0.24 to 0.38 γ per milliliter of aureomycin and heated at 160°, 185°, 200°, and 210° F. until the aureomycin was inactivated completely. Average results of three to four replicate trials are presented (Figure 1). Thermal inactivation of aureomycin in milk resulted at 160°, 185°, 200°, and 210° F. for an average of 280, 90, 50, and 30 minutes, respectively. Time required for the complete inactivation of aureomycin at each temperature studied did not vary greatly (a maximum of $\pm$ 9% deviation from the average) in different samples of milk. Nor did the different concentrations of aureomycin used in these trials materially vary the time required for its inactivation. A linear relationship was observed when the results were plotted as logarithm of time versus temperature. The Z value of aureomycin curve was calculated to be 51.5.

Effect of various heat treatments on aureomycin. To determine the effect of various heat treatments for different lengths of time upon aureomycin, raw milk, 0.1 *M* mono-potassium phosphate buffer (pH 4.7), and water were inoculated with 0.2 to 1.0 γ per milliliter of aureomycin. The samples were mixed thoroughly, dispensed into screw-capped test tubes, heated at 143° and 160° F. for 10, 20, 30, 40, and 50 minutes, and cooled. Following the heating, the samples were promptly assayed for the antibiotic by the disk assay technique. Data relative to the rate of aureomycin inactivation are presented (Table 1). In general, the data revealed that upon heat treatment the antibiotic lost its potency at a faster rate in buffer than in milk, and fastest in water. Also, the loss of aureomycin was directly related to the heat treatment. Similar studies were con-

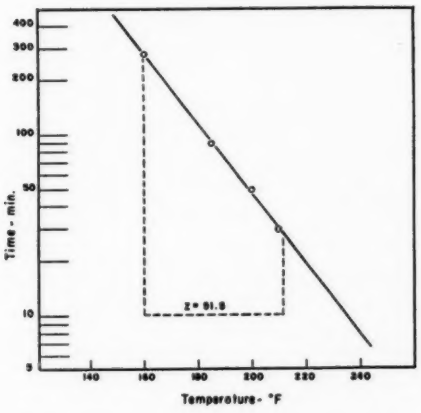

FIG. 1. Thermal inactivation curve for aureomycin in milk.

ducted to determine the potency loss in milk, buffer, and water when heated at 250° F. in an autoclave for 5, 10, and 15 minutes. Results revealed that the antibiotic was inactivated completely within five minutes in all three media.

Effect of heat and storage upon loss of aureomycin. To determine the loss of aureomycin activity upon heating and during storage of unheated and heated systems, raw milk, phosphate buffer, and water were inoculated with concentrations of from 0.30 to 0.62 γ of aureomycin per milliliter. Separate samples were prepared for the study of each temperature. Each medium was divided into two lots. One lot was heated and the other was raw. Antibiotic assays were run immediately on the raw and processed samples. They were then stored at 34° to 38° F. and assayed every week for five weeks.

The antibiotic stability curves, plotting per cent of aureomycin inactivation against time for the three media, are presented (Table 2 and Figure 2). The curves represent average results of four to eight trials. Loss of aureomycin was directly related to the heat treatment and was in an increasing order in milk, buffer, and water. Heating of the three media containing an average of 0.45 γ aureomycin per milliliter at 143° F. for 30 minutes produced 13.0 to 22.0%, with

TABLE 1

Loss of aureomycin potency in milk, buffer, and water upon heat treatment

Minutes	143° F.			160° F.		
	Milk	Buffer	Water	Milk	Buffer	Water
	(%)					
10	9.3	9.9	14.0	13.5	17.2	24.2
20	14.2	18.0	23.7	20.1	31.6	46.3
30	18.4	23.3	36.7	27.1	37.4	56.1
40	22.2	31.8	40.3	32.5	47.4	79.3
50	25.4	38.9	48.3	38.2	55.1	100.0

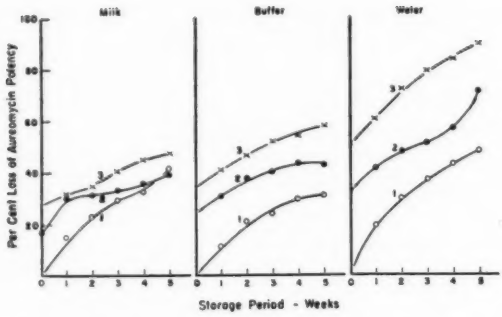

FIG. 2. Effect of heat treatment and storage time on the loss of aureomycin potency in milk, buffer, and water. 1 = unheated; 2 = heated at 143° F. for 30 min.; 3 = heated at 160° F. for 30 min.

an average of 16.6%, loss of aureomycin in milk; 16.7 to 30.0%, with an average of 24.2%, loss in buffer, and 23.4 to 41.3%, with an average loss of 32.5%, in water. Heating at 160° F. for 30 minutes produced a comparatively greater loss of aureomycin in the three media than was obtained at 143° F. It was observed that after heating, the three media still contained antibiotic activity equivalent to 0.30 to 0.38 γ per milliliter in the samples heated at 143° F., and 0.19 to 0.28 γ per milliliter in the samples heated at 160° F. During storage for five weeks, 41.4% of the aureomycin was lost in the raw milk samples. In the heated milk samples total loss upon heating and storage was 39.6% in the samples heated at 143° F., and 47.1% in the samples heated at 160° F. In the buffer series, 30.6% aureomycin was lost in the unheated samples; whereas, the loss upon heating and storage was 42.5 and 57.9% in the samples heated at 143° F. and 160° F., respectively. In the water samples, the pattern of losses in the unheated and heated samples was observed to be similar to that in the milk and buffer series, except that the loss was greater in the water than in two other systems. The per cent inactivation of aureomycin due to heat and storage varied slightly in different trials with milk, buffer, and water. However, the concentration of the antibiotic used in these studies and the extent of inactivation upon heating and storage showed no relationship.

In the unheated samples and in those heated at the lower temperature, the storage loss was of an increasing order in buffer, milk, and water; whereas, in the samples heated at 160° F. the storage loss was less in milk than in buffer and the most loss occurred in water. The total loss upon heating at 143° F. or 160° F. and storage was greater in buffer and water than in milk. This was because of a considerably greater inactivation of aureomycin in buffer and water upon heating. After five weeks of storage, both unheated and heated samples still possessed detectable antibiotic potency.

During storage, 41.4% of the aureomycin was lost in raw milk and only 19.5 to 23.0% in the heated samples. In the buffer and water systems, the difference

TABLE 2

Per cent loss of aureomycin activity in milk, buffer, and water upon various heat treatments and storage

Heat treatment	No. of trials	Aureomycin inoculated	Loss upon heat treatment			Loss during storage for five weeks			Total loss upon heating and storage		
			Milk	Buffer	Water	Milk	Buffer	Water	Milk	Buffer	Water
		(γ/ml)	(%)								
Raw	8	0.30–0.51	—	—	—	41.4	30.6	47.5	41.4	30.6	47.5
143° F. for 30 min.	4	0.30–0.51	16.6	24.2	32.5	23.0	18.3	38.8	39.6	42.5	71.3
160° F. for 30 min.	4	0.30–0.62	27.6	34.3	51.2	19.5	23.6	38.1	47.1	57.9	89.3
250° F. for 15 min.	2	0.40–0.50	100.0	100.0	100.0	—	—	—	100.0	100.0	100.0
(Autoclaving at 15 lb. for 15 min.)											

between the storage losses in unheated and heated samples was not as wide as in the milk.

DISCUSSION

Results presented (Figure 1) are of significance to the food industry. The preparation of a curve of this nature would make possible theoretical calculation of various heat treatments required for inactivation of aureomycin when present in milk.

The Z value for aureomycin in milk was 51.5, as compared to 49.0 for penicillin, reported previously. It is interesting to note that although the Z values for the two antibiotics differ very little, there was a great variation in the heat treatments necessary to inactivate the two antibiotics. A heat treatment of 160° F. for 280 minutes was required to inactivate aureomycin; whereas, 1,705 minutes were required to inactivate penicillin at the same temperature.

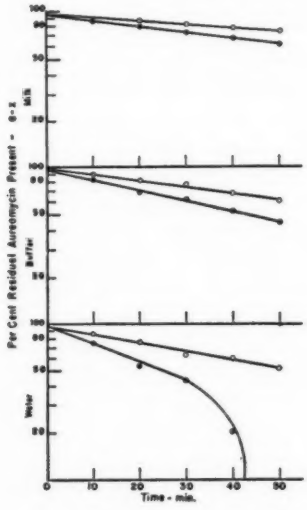

FIG. 3. Thermal inactivation of aureomycin in milk, buffer, and water at 143° F. and 160° F. –o– = heated at 143° F.; –●– = heated at 160° F.

In order to determine whether or not the rate of heat inactivation of aureomycin against time was of the first order, the differential equation suggested by Prutton and Maron *(7)* was applied to the data in Table 1; results have been plotted in Figure 3. The equation is as follows:

$$\text{Log } (a - x) = \frac{-k_1}{2.303} t + \log a$$

where a = initial concentration, x = loss, t = time, and k is a constant. Since a is constant, this equation will yield a straight line when log $(a - x)$ is plotted against time, if the equation is of the first order. In Figure 3, log $(a - x)$ has

been plotted against time. It was observed that the rate of inactivation of aureomycin was of the first order when heated at 143° F. and 160° F. in milk or buffer and at 143° F. in water. The few points that fell outside the straight lines were within the experimental error. In water at 160° F., however, the reaction was not of the first order, as the plot did not follow a straight line.

In this study during storage, aureomycin inactivation occurred faster in raw samples than in the heated ones. In a related study with streptomycin *(9)*, it was observed that an appreciable loss of the antibiotic occurred during storage in raw milk and that no loss occurred in sterilized milk. Also, it was observed that terramycin loss during storage was faster in broth containing the larger microorganism inoculum and in broth incubated at the higher temperature *(8)*. On the basis of the above information, it is suggested that naturally present heat-labile microorganisms and/or enzymes might be partially responsible for inactivating aureomycin at a faster rate in raw milk than in the heated samples.

The findings that aureomycin activity decreased during storage of milk, and that it was comparatively more stable in the phosphate solution at pH 4.7 than in water, are corroborated by the findings of previous workers, who have reported that blood serum and various culture media antagonized aureomycin activity *(3)* and that the potency decreased at a faster rate as the pH of the medium was increased from 5.1 to 8.0 *(6)*.

In general, on the basis of previous work *(9, 10)*, aureomycin seems to be comparatively more heat-labile than penicillin and streptomycin. In contrast, aureomycin is apparently less susceptible to storage losses than are the two other antibiotics.

SUMMARY

The stability of low concentrations of aureomycin in milk, phosphate buffer, and water has been determined by the disk assay method, by using brain heart infusion agar as the assay medium and *Bacillus cereus* as the test organism.

The complete thermal inactivation curve for aureomycin in milk has a Z value of 51.5.

Milk, buffer, and water inoculated with aureomycin were heated at 143°, 160°, and 250° F. for various lengths of time. The results revealed a direct relationship between the heat treatment and extent of aureomycin inactivation. Also, aureomycin lost its potency in an increasing order in milk, buffer, and water. The rate of aureomycin inactivation was of the first order when heated at 143° F. and 160° F. in milk and buffer, and at 143° F. but not at 160° F. in water. At 250° F., the antibiotic was inactivated completely within five minutes in all three media.

Aureomycin lost its activity in milk, buffer, and water during storage. In the unheated samples and in the samples heated at 143° F. for 30 minutes, the storage loss was less in buffer than in milk and the most loss occurred in water. In the samples that were heated at 160° F., the storage loss was less in milk than in buffer; the most loss occurred in water. Also, the rate of loss of aureomycin during storage was slower in heated than in unheated samples.

Heating at 143° F. or 160° F. for 30 minutes, with or without prolonged storage, did not completely inactivate aureomycin in milk, buffer, or water. The antibiotic did not lose its activity completely in raw milk, buffer, or water during storage.

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CERTAIN FACTORS AFFECTING THE CONCENTRATION OF ACTIVE SULFHYDRYL COMPOUNDS IN HEATED CREAM[1]

I. A. GOULD AND P. G. KEENEY[2]
Department of Dairy Technology, The Ohio State University, Columbus

Cream destined for frozen storage is heated to higher temperatures than that processed for market purposes, in order to produce reducing compounds that protect it against oxidative changes. Evidence is presented to show that temperatures higher than those used in industry are more effective in liberating reducing groups in cream. In the light of this work, a reappraisal of industry practice is timely. *Editor.*

High-temperature heat treatment is accepted as an important step in processing cream that is to be stored subsequently in the frozen state. It is recognized that such heat treatment produces active sulfhydryl (-SH) compounds, which serve as antioxidants *(1, 4, 5, 9)*. However, only limited specific attention has been given to the times and temperatures of heating necessary to produce the maximum concentration of these reducing groups. It was to obtain additional information in this connection that the present study was conducted.

EXPERIMENTAL PROCEDURE

Unless specified otherwise, cream was obtained by cold-separation ($< 50°$ F.) of fresh mixed-herd milk, and the 45-50% obtained cream was standardized to 40% with the skimmilk obtained from the separation. Milk and cream were handled at all times in such a manner as to prevent contamination with metals.

Heating of the cream was conducted in a laboratory flash-heating apparatus, shown in Figure 1. This apparatus was designed in two sections: a heating unit and a cooling unit. The heating apparatus consisted of 16 ft. of ¼-in. glass tubing immersed in a 17 in. × 13 in. × 10 in. water bath. All joints and valves were ground glass. The temperature of the water in the bath was thermostatically controlled to within $\pm 1.0°$ F. The cooling unit was similarly constructed. The apparatus was designed and operated so that 2 liters of cream were forced through the closed system in 30 seconds and the time required for the cream to reach 190° F. was 30 seconds. A thermometer, inserted into the tubing near the exit end of the system, insured accurate control of the heating temperature.

When the cream was to be held for various time-intervals at the processing temperature, it was diverted into 13 × 100 mm. Pyrex test tubes by means of a

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[2] Present address: The Pennsylvania State University.

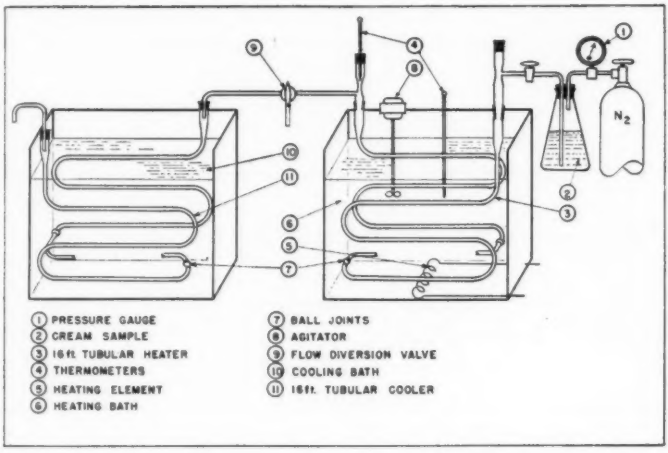

FIG. 1. Diagrammatic sketch of heating unit used.

3-way valve located in the system at the point where the cream left the heating apparatus. These test tubes were placed immediately in a thermostatically controlled water bath, held at the desired temperature for the proper length of time, and then transferred to ice-water for cooling.

A modification of Harland and Ashworth's thiamin disulfide (TDS) method was used to measure quantitatively the active -SH groups produced *(1)*. The various reagents were prepared in accordance with Harland and Ashworth's directions *(6)* and their general recommendations were followed throughout. The procedure used for measuring the thiamin disulfide reducing materials in cream follows: A 2-g. sample was weighed into a 13 × 100 mm. Pyrex test tube. One ml. of the thiamin disulfide reagent was added and the contents of the tube were mixed thoroughly. Three drops of isobutanol were added to prevent interference from the air. The samples were incubated at room temperature for 2 hours, after which 2 ml. of a 10% trichloroacetic acid solution was added to precipitate the protein. The sample was then filtered through a No. 1 Whatman filter paper and 1 ml. of the filtrate was made up to a volume of 50 ml. with distilled water.

Two ml. of this mixture was pipetted into a 20 × 150 mm. test tube and the thiamin content of this portion was determined. To accomplish this, 3 ml. of distilled water and 15 ml. of isobutanol were added to the test tube, and the two layers were mixed by using an ebullition tube to bubble compressed air through the medium. The thiamin was oxidized to thiochrome by 2 ml. of the potassium ferricyanate solution. After 2 minutes, the ebullition tube was removed, and the bottom layer was siphoned off. Water, trapped in the alcohol, was removed by the addition of 1-2 g. of anhydrous sodium sulfate. After centrifuging to facilitate settling of the sodium sulfate, the thiochrome-containing alcohol was transferred to matched cuvettes and the fluorescence was measured

with a Model 12 Coleman Photofluometer. The readings were referred to a standard curve for conversion to thiamin values. Blank values were obtained for heated cream by adding trichloroacetic acid before the addition of thiamin disulfide.

Inasmuch as heat treatment makes available -SH groups for measurement by the TDS method, the expressions "heat-activated," "heat-revealed," or "active" are used throughout to indicate these groups. This is done to avoid confusion with the -SH values obtained by means of the orthoiodosobenzoate method *(10, 11)*.

EXPERIMENTAL RESULTS

Time-temperature. The first experiment was conducted to determine the effect of different heating times and temperatures on the quantity of active -SH compounds produced in 40% cream. Temperatures of 160°, 170°, 180°, and 190° F. for varying holding times up to 30 minutes were used. The results are shown in Figure 2.

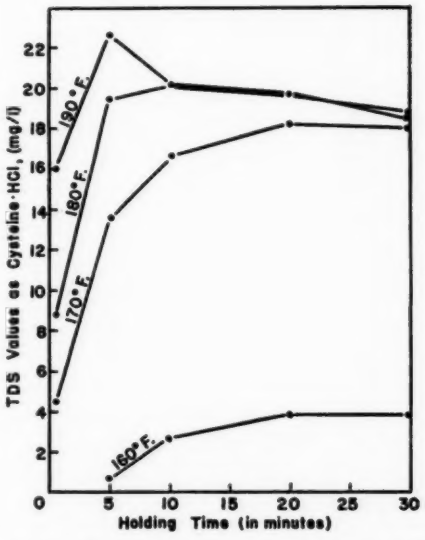

FIG. 2. Effect of different heating temperatures and times on the concentration of sulfhydryl compounds of 40% cream.

This figure reveals a direct relationship between temperature of heating and quantity of -SH compounds. For the higher temperatures, the maximum production of active -SH compounds occurred within a rather narrow limit of heating time. For example, the 190° F. curve shows the highest -SH group concentration of any of the temperatures used when the holding time was for 5 minutes. However, prolongation of the holding period caused a marked decrease in the quantity of the -SH compounds, and the 190° F. values became

essentially the same as those for 180° F. at the 10-minute period. These results indicate that to obtain the maximum active -SH compound concentration for 170° F., 180° F., and 190° F., the holding times necessary are 20 minutes, 10 minutes, and 5 minutes, respectively.

Seventy-two per cent of the active -SH compounds that were in the cream after 5 minutes at 190° F. had been produced during the flash-heating period. This value of approximately 16 mg. per liter cysteine · HCl was approximately twice the concentration created during flash-heating at 180° F. and was almost four times that formed during flash-heating at 170° F.

Fat level and heating conditions. A study was conducted to determine the effect of different levels of fat in the cream on the quantity of heat-exposed -SH compounds by processing 20%, 30%, 40%, and 50% cream. Results are shown in Figure 3.

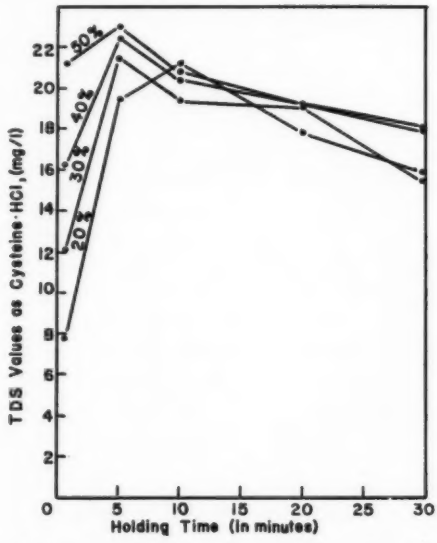

FIG. 3. Effect of variations in the fat content of cream on the concentration of sulfhydryl compounds revealed by heating at 190° F. for various times.

These results reveal that the higher the fat content of cream the greater was the quantity of the active -SH compounds produced by heating at 190° F. for short periods. Furthermore, the higher the fat content of the cream, the more rapidly were the active -SH compounds created. Ninety-five per cent of the maximum -SH concentration of the 50% cream was revealed during the 30-second exposure. The 190° F. flash-heating resulted in the 50% cream having active -SH concentrations about three times those of the 20% cream and about twice those of the 30% cream.

As the holding periods were extended, the -SH values for all the creams became more nearly the same, and after a 30-minute exposure the sulfhydryl

concentrations in the 40% and 50% creams were only about 2 mg. per liter greater than those in the 20% and 30% creams. This may indicate that the material supplying the major share of the -SH compounds is in a more heat labile condition in the higher fat testing cream but is not necessarily present in larger quantity than in the lower fat creams.

Except for the 20% cream, the data indicate that regardless of the fat level, 5 minutes at 190° F. yielded the maximum active -SH values for this temperature. Ten minutes at 190° F. was required to produce the greatest quantity of active -SH compounds in the 20% cream. Extending the heating time beyond that necessary for maximum -SH release caused a loss of TDS-reducing substances regardless of the fat level.

Effect of rate of heating. A study was conducted to determine the effect of the rate of heating on active -SH compound production. This was accomplished by adjusting the heating system so that the times required for the cream to reach 190° F. were 15, 30, and 60 seconds. As noted previously, the standard procedure used in this study involved a 30-second rate of heating. Although the complete data are not presented, they revealed -SH values of 12.9, 16.4, and 20.5 mg. per liter cysteine · HCl for the 15, 30, and 60-second periods, respectively. These results indicate that varying the time to heat the cream to 190° F. in the continuous system materially affected the quantity of active -SH compounds produced. The 60-second heating time yielded a concentration of active -SH compounds approximately 90% of that for cream held 5 minutes at 190° F. The increased production of active -SH groups by the longer "heat-up" time would appear to represent greater exposure of the milk within the critical temperature range. It is of interest to note that under the processing conditions used, major alterations in the concentration of the heat-revealed -SH groups are produced in a matter of seconds in heating time.

Effect of aeration and nitrogen aspiration. Since some commercial processing of cream may involve the inclusion of air prior to subjecting the cream to heat treatment, it seemed desirable to determine whether such air incorporation would affect the concentration of TDS-reducing substances produced by heating at 190° F. Therefore, 40% cream was divided into two lots. Lot 1 was forced through the flash-heating apparatus with nitrogen and Lot 2 with compressed air. Preliminary to heating, nitrogen was bubbled through the former cream portion for 5 minutes and compressed air through the latter for a similar period.

The concentrations of sulfhydryl compounds produced by heating at 190° F. for 5 minutes were 21.3 and 22.0 mg. per liter cysteine · HCl for the aerated and the nitrogen-pressurized creams, respectively. The slight difference noted was consistent for various trials and for different time-temperature combinations used, varying between 0.5 and 1.2 mg. cysteine · HCl per liter. Therefore, these results indicate that in comparison to the nitrogen-treatment the aeration did not influence materially the concentration of active -SH compounds produced by the 190° F. heat treatment. This suggests that, in the commercial handling of cream, the inclusion of air prior to heat treatment will not affect the potential antioxidant property which will result from the subsequent heat treatment.

Effect of separating temperature. It has been suggested that the adsorbed layer is different in composition and characteristics for solid and liquid fat globules *(12, 13)*. If the chemical composition of such films varies in respect to the potential quantity of heat-activated -SH compounds, then the temperature of separation of the milk would have practical significance in producing cream which would have the maximum antioxidant property as the result of heat treatment. To determine whether or not such a situation exists, the following experiment was undertaken: Cold milk (40° F.) that had been undisturbed for at least 6 hours was divided into three lots. One lot was adjusted to 55° F., another to 100° F., and the third to 135° F. Each lot of milk was separated at its specified temperature and the cream was standardized to 40% fat with the skimmilk obtained from that separation. The resulting creams were heated to 190° F. and held for varying lengths of times at that temperature. Results are presented in Figure 4.

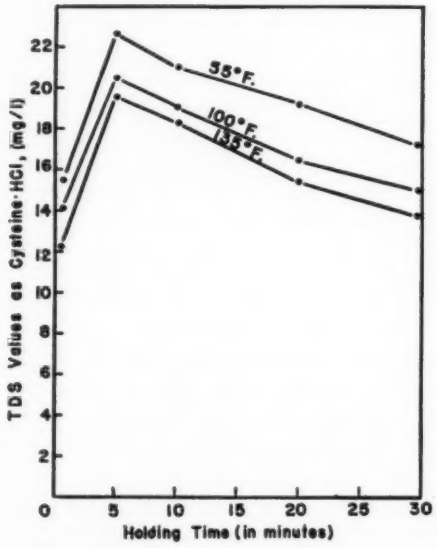

FIG. 4. Influence of separation temperature on the subsequent production of active sulfhydryl compounds in cream heated to 190° F. for various times.

The results reveal a definite inverse relationship between the temperature of separation and the amount of heat-activated -SH compounds. The differences in the concentrations of the active -SH compounds in the three creams were consistent at all holding times. At corresponding holding times for 190° F., cream separated at 135° F. exhibited 14-20% (2.6-3.8 mg. per liter cysteine · HCl) less active -SH compounds than the cream separated at 55° F.

Effect of storage. Investigators have observed that the cooked flavor imparted to cream pasteurized at high temperatures diminishes during frozen storage *(2)*,

a change which may be related to a decrease in concentration of the active -SH compounds.

To determine the extent of this change during storage, 40% cream was heated to 190° F. and collected in a series of 13 × 100 mm. Pyrex glass test tubes. The tubes were stoppered tightly and held for 5 minutes at 190° F., after which they were cooled quickly and divided into two sets. One set was stored at 5° F. and the other at 40° F. At periodic intervals, one tube of cream from each temperature was removed from storage and analyzed. Results are presented in Figure 5.

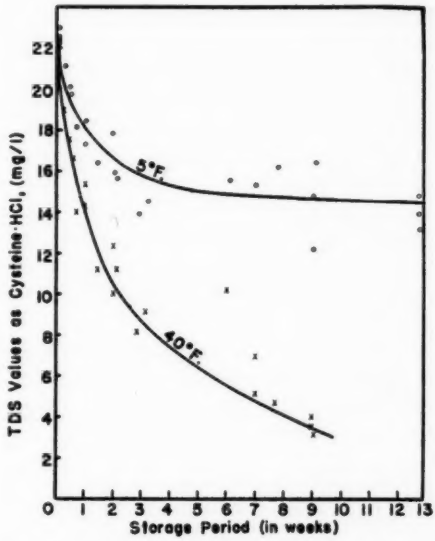

FIG. 5. Influence of storage temperature on the stability of sulfhydryl compounds in cream heated at 190° F. — 5 min.

During the early stages of storage, the creams at both 5° F. and 40° F. underwent rather rapid depletion. Twenty-five per cent (4.5-6.6 mg. per liter cysteine · HCl) of the active -SH compounds disappeared during the first 2 weeks storage of the cream at 5° F., whereas approximately 56% had disappeared in the cream at 40° F. The concentration of active -SH groups in the cream stored at 5° F. tended to level off after about 2-4 weeks of storage and, on the average, about 67% of the original -SH group concentration remained in the cream at the end of 13 weeks of storage. In contrast, the cream held at 40° F. exhibited a continuing loss of the active -SH compounds and approximately 17% remained at the end of 8 weeks of storage.

The data in Figure 5 indicate that the results for the three trials varied appreciably and the curves as presented depict general trends rather than actual values.

A study was also conducted to determine whether drastic aeration of the cream prior to heat treatment might affect the loss of the active -SH compounds in the cream during frozen storage. In this experiment, 40% cream was aerated vigorously, forced through the flash-heating apparatus with compressed air, heated at 190° F. – 5 minutes, cooled, and stored at 5° F. A non-aerated lot served as the control. The -SH compounds were measured periodically. The results revealed that at the end of 40 days of storage, the average -SH content of the control and the aerated lots represented 14.6 and 14 mg. per liter cysteine · HCl, respectively. Thus, the aeration did not accelerate loss of the active -SH compounds.

Effect of metal contamination. Experiments were conducted to determine the relationship between copper-induced oxidized flavor and the stability of heat-revealed -SH compounds in frozen cream during storage. In the initial study, the effect of copper contamination after high-temperature pasteurization was investigated. Forty per cent cream was processed at three different time-temperature levels: 190° F. flash; 190° F. – 5 minutes; 170° F. – 20 minutes. The cream was divided into four lots. Lot 1 served as the control, Lot 2 contained 1 p.p.m. of added copper (as aqueous copper sulfate), Lot 3 contained 1 p.p.m. of copper plus 1 p.p.m. of manganese (as aqueous manganese sulfate), and Lot 4 contained 1 p.p.m. of manganese. Manganese was included in these studies because this ion has been reported to be capable of retarding copper-induced oxidized flavor in milk *(3)*.

Results presented in Table 1 corroborate previous findings that the addition of copper after pasteurization causes an immediate and marked decrease in the active -SH compounds of the cream. The 1 p.p.m. of copper destroyed approxi-

TABLE 1

Effect of adding copper and manganese singly and in combination after heating on the loss of active –SH compounds of cream during storage. (Values expressed as mg/l of cysteine · HCl)

Treatment of cream	Storage period (in days) at 5° F.				
	0	2	7	19	35
	(mg/l)				
190° F., flash					
Control	14.9	12.2	12.5	11.8	12.2
1 p.p.m. Copper	7.3	6.4	5.0	2.3[x]	2.0[x]
1 p.p.m. Copper and 1 p.p.m. manganese	6.4	5.5	5.0	2.3[x]	2.1[x]
1 p.p.m. Manganese	13.4	10.8	12.5	9.9	9.7
190° F., 5 min.					
Control	23.0	16.6	16.0	16.3	15.5
1 p.p.m. Copper	13.1	10.8	8.2	3.2	3.0[x]
1 p.p.m. Copper and 1 p.p.m. manganese	11.4	9.0	5.8	3.2	3.0[x]
1 p.p.m. Manganese	21.3	14.3	17.8	15.2	14.0
170° F., 20 min.					
Control	18.7	15.2	15.2	13.4	11.4
1 p.p.m. Copper	11.4	7.3	6.5	2.9[x]	2.0[x]
1 p.p.m. Copper and 1 p.p.m. manganese	10.5	7.3	6.4	3.0[x]	2.3[x]
1 p.p.m. Manganese	16.9	12.5	13.4	13.3	10.5

[x] = Oxidized flavor.

mately 50%, 43%, and 39% of the active -SH compounds produced by 190° F. flash, 190° F. – 5 minutes, and 170° F. – 20 minutes, respectively. During frozen storage, the decrease in the concentration of -SH compounds in copper-contaminated cream was accelerated in comparison to the controls. An oxidized flavor was detectable when the concentration of thiamin disulfide reducing substances was equivalent to about 3.0 mg. per liter cysteine · HCl or less. The higher the initial active -SH value, the slower was the oxidized flavor development. The manganese was apparently without effect in the concentrations used.

Another experiment was conducted to study the effect of the addition of copper or manganese prior to high-temperature pasteurization on the heat-activated -SH compounds. Conditions were the same as described for the previous series. The results are shown in Table 2.

TABLE 2

Effect of metal contamination prior to the heat treatment on the loss of active –SH compounds of cream during storage. (Values expressed as mg/l of cysteine · HCl)

Treatment of cream	Storage period (in days) at 5° F.				
	0	2	7	19	35
	(mg/l)				
190° F., flash					
Control	13.1	11.4	12.2	8.2	9.0
1 p.p.m. Copper	11.4	8.7	7.0	3.8	3.5
1 p.p.m. Mn	14.3	11.7	11.4	8.7	7.9
190° F., 5 min.					
Control	23.0	20.1	19.2	16.6	15.5
1 p.p.m. Cu	14.3	9.6	7.6	6.4	3.8
1 p.p.m. Mn	23.0	19.2	19.5	14.0	16.0
170° F., 20 min.					
Control	18.3	16.0	15.0	13.4	11.6
1 p.p.m. Cu	13.3	8.0	5.2	1.2[x]	2.0[x]
1 p.p.m. Mn	16.3	14.6	11.4	6.4	7.6

[x] = Oxidized flavor.

The results reveal that the initial active -SH concentration in the copper-contaminated cream was considerably less than that of the control and that containing manganese, but was greater than the copper-containing cream (Table 1).

During storage, the active -SH concentration dropped rapidly in those creams containing copper, and the rate of loss was similar to that of the copper-containing cream (Table 1). However, in these trials, no oxidized flavor was detectable after 35 days of storage except in the copper-contaminated cream pasteurized at 170° F. for 20 minutes. The active -SH group concentration was again low when oxidized flavor was detected.

The manganese was generally without effect in influencing the initial concentration of active -SH compounds or their subsequent changes during storage. However, the -SH group values were lower in the manganese-containing cream than in the control in the series heated to 170° F. for 20 minutes.

The results in Tables 1 and 2 emphasize the value of high initial active -SH concentration by the fact that, at the end of the 35-day storage period, the

-SH concentration in the control was appreciably greater in the lot heated to 190° F. for 5 minutes than in the two other lots. Also, the active -SH values for the controls for 170° F. treatment are larger than for the 190° F. flash samples. These results are in confirmation of those illustrated by Figures 2 and 3.

DISCUSSION

The importance of temperature and exposure-time in the production of a maximum quantity of sulfhydryl (-SH) compounds measured by the thiamin disulfide (TDS) method is emphasized by the findings in this study. The 30-second heating process utilized as the standard created rather wide differences in the active -SH compound concentrations in the creams heated at the different temperature levels. In fact, there was an approximate linear relationship between the flash-temperature used and the active -SH compound concentration at the temperatures studied. At the higher temperatures, a 10° F. rise in temperature was accompanied by a doubling of the active -SH compounds. Thus, 180° F. produced twice as much TDS-reducing capacity as 170° F., and 190° F. was four times as effective as 170° F. in this respect.

In commercial practices, frequently recommendations are made that cream processed for frozen storage should be heated to 170° F. for a 20-minute period. The results in this study indicate that a reappraisal of these recommendations is in order and that higher temperatures and shorter exposure times may be utilized to advantage.

The question is not answered as to the cause for the rather rapid decrease in concentration of active -SH compounds with a relatively short extension of the heating time when the higher temperatures were used. The results in this regard are similar to those reported by Harland and Ashworth *(6)* and Harland, Coulter, and Jenness *(7)*. Other work indicates that, under normal conditions, the decrease in TDS-reducing substances in milk which occurs almost immediately after the heating temperature is reached involves the volatilization of hydrogen sulfide *(1)* and oxidation *(8)*. However, in this present study, the cream exposed for prolonged periods was held in small, tightly stoppered containers to reduce the losses due to these sources. Even so, the decrease in TDS-reducing substances was of a considerable magnitude.

The temperature of separation of cream for possible frozen storage cannot be ignored as having an influence on the quantity of heat-activated -SH compounds. The possibility exists that *(a)* the -SH contributing proteins are affected by the higher separation temperatures and will not be as susceptible to -SH group formation by heat as are those in cream separated from cold milk or *(b)* separating the fat in the liquid condition causes a loss in the concentration of the fat-associated material that yields the heat-revealed -SH compounds. It has been shown that "the agglutinating substance" (euglobulin) remains in the cream separated from cold milk but is thrown into the skimmilk when cream is separated at temperatures high enough to liquefy the fat *(13)*.

Although abnormally vigorous aeration of the cream before heating did not materially alter the concentration of the heat-produced TDS-reducing materials

nor their loss in the cream during a relatively short period of frozen storage, aeration may produce certain incipient oxidative effects in the cream which may become more noticeable upon prolonged storage periods. However, the data obtained would indicate that the small amount of air incorporated in cream in a commercial operation prior to heat processing is not of major concern from the standpoint of subsequent keeping properties of the cream as they are related to active -SH group stability.

Not only do the studies involving copper contamination corroborate earlier findings dealing with the time of adding the copper, i.e., whether before or after heating, but they indicate the importance of obtaining a maximum amount of active -SH compounds in the cream during heat treatment to prevent subsequent development of oxidized flavor. In other words, the results prove that processing cream at 190° F. for 5 minutes is definitely preferred to 170° F. for 20 minutes for maximum stability to oxidative changes.

SUMMARY

A study was conducted to determine the effect of the following factors on the concentration of active sulfhydryl (-SH) compounds in heated cream: *(a)* temperature of heating and length of exposure, *(b)* fat concentration, *(c)* separating temperature, *(d)* aeration, *(e)* length and temperature of storage, *(f)* metal contamination. The thiamin disulfide (TDS) method was used to measure the concentration of the -SH compounds. The cream was heated in a specially constructed tubular-heating apparatus in which heating time was controlled usually at 30 seconds.

The greatest concentrations of active -SH compounds produced in 40% creams that were heated to 190° F., 180° F., and 170° F. were formed during periods of 5, 10, and 20 minutes, respectively. The maximum concentration was achieved by heating the cream to 190° F. for 5 minutes. When the rates of heating the cream to 190° F. were 15 seconds, 30 seconds, and 60 seconds, the quantities of active -SH compounds produced were directly and markedly increased.

A direct relationship was noted between fat levels of cream and active -SH group production when creams of 20%, 30%, 40%, and 50% fat were flash-heated. Flash-heating to 190° F. produced in 50% cream three times the quantity of active -SH compounds as that produced in 20% cream, and twice that formed in 30% cream. However, as the exposure periods were extended to 5 minutes or more, the values became more nearly the same.

The temperature of separation of milk was related inversely to the concentration of heat-revealed -SH compounds in the resulting cream. The concentrations of active -SH compounds in 40% cream heated to 190° F. for 5 minutes were equivalent to 22.7, 20.5, and 19.6 mg. per liter cysteine · HCl when the separation temperatures were 55° F., 100° F., and 135° F., respectively.

Vigorous aeration prior to the high-temperature processing did not affect significantly the concentration of heat-activated -SH compounds nor the stability of the compounds during subsequent storage of the cream.

About 60-70% of the heat-activated -SH compounds were retained in cream stored for 13 weeks at 5° F., but only 10-20% were retained in cream stored 9 weeks at 40° F. Addition of 1 p.p.m. copper to the cream before or after heating accelerated loss of the active -SH compounds during storage at 5° F., but the addition of manganese in the same concentration as the copper was without effect. Oxidized flavor occurred in the copper-containing cream when the concentration of active -SH compounds had decreased to a level approximating 3 mg. per liter cysteine · HCl.

The commercial significance of these findings is discussed.

ACKNOWLEDGMENT

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LACTOSE CRYSTALLIZATION IN ICE CREAM. III. MODE OF ACTION OF MILK POWDER IN PREVENTING SANDINESS

T. A. NICKERSON

Department of Dairy Industry, University of California, Davis

Lactose crystallization proceeds at the same rate in ice cream containing milk powder as it does in seeded ice cream. The lactose of the powder supplies crystal nuclei that serve as seed to form undetectable crystals in partially frozen ice cream which may be saturated with lactose. *Editor.*

A method of producing ice cream of high serum solids without risking sandiness was patented by Gray and Turnbow *(2)* in 1932. The method consists of adding milk powder to partially frozen mix in the freezer. The generally accepted explanation *(6)* has been that the lactose of the powdered milk does not dissolve, because the partially frozen mix is saturated with respect to lactose. Therefore, the ice cream has the same lactose concentration as a low serum solids product, which does not tend to go sandy.

In the light of recent work *(4, 5)*, another explanation seemed possible. A number of workers *(1, 3, 6)* using this method have noted that extremely large numbers of small crystals developed in the ice cream during storage. This suggested that the addition of milk powder to the mix produced numerous crystal nuclei and that these acted as seeding material. The supersaturated lactose would be deposited in the form of undetectably small crystals, which would eliminate the danger of sandiness, as in a seeded ice cream *(4)*.

The purpose of this study was to test the two proposed theories on the mode of action of milk powder in preventing sandiness in ice cream. If the accepted explanation is valid and the lactose of the milk powder does not go into solution, then the lactose crystallization would be slow, as in a normal low-solids ice cream. On the other hand, if the milk powder caused the development of numerous crystal nuclei, the lactose crystallization would be rapid *(5)*, as in a seeded ice cream.

EXPERIMENTAL PROCEDURE

Composition of the mixes was 12% fat, 14% M.S.N.F., 15% sucrose, and 0.3% stabilizer. Cream was used as the source of fat, and condensed skimmilk was used as the milk solids-not-fat concentrate, except that 4% nonfat dry milk solids was added to some mixes to increase the solids-not-fat from 10% to 14%. The processing procedure has been described previously *(4)*.

Three types of ice cream were prepared: A, a normal ice cream, served as a control; B was seeded during freezing with fine edible whey powder at the rate

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of four g. per gallon of mix; C had the final 4% M.S.N.F. added in the form of regular spray-dried nonfat dry milk solids after the mix had started to freeze.

The ice creams were hardened at –10° F. or below for 24 hours, and samples were stored at approximately 2° and 12° F. At intervals, samples were removed from storage and examined microscopically and polarimetrically, as previously described *(4, 5)*, for lactose crystallization.

RESULTS

Data in Table 1 show the rates of lactose crystallization (as shown by the increasing percentage of the alpha form) in the three types of ice cream. The normal ice cream showed only a small increase in the percentage of alpha lactose

TABLE 1

Percentages of alpha lactose in normal ice cream, seeded ice cream,[a] and ice cream containing milk powder,[b] during storage at 2° and 12° F.

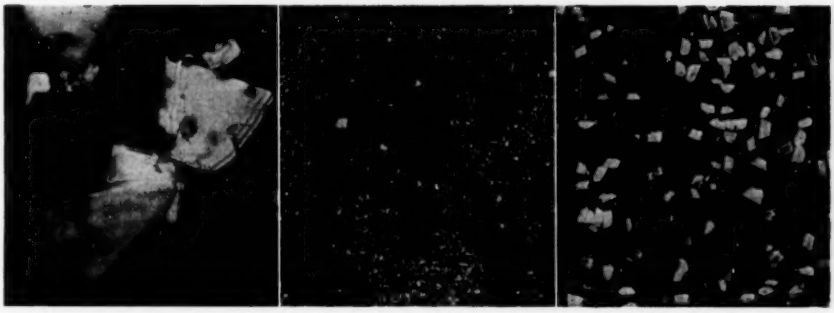
Storage	2° F.			12° F.		
	Normal	Seeded	Milk powder	Normal	Seeded	Milk powder
(days)	(%)					
½				35.6	39.0	36.7
1				37.9	49.5	49.2
4	37.5	61.5	45.6	38.6	75.9	72.2
5				38.4	76.3	74.4
6	36.0	70.6	61.0	35.6	81.7	81.0
7				35.7	82.7	80.1
8	38.4	76.0	67.1	36.0	85.9	82.8
11				37.7	87.6	85.2
12				38.2	89.5	86.2
21					89.7	92.5

[a] Four g. fine whey powder added per gallon mix during freezing.
[b] NFDMS equivalent to 4% serum solids added during freezing.

during the storage period. The seeded ice cream and the ice cream containing the milk powder, however, showed a rapid increase in the percentage of alpha. Moreover, the rates at which alpha increased in these two ice creams were quite similar. Repetition of the entire experiment gave the same type of result, and confirmed that lactose crystallized at essentially the same rate in the seeded ice cream and in the ice cream to which milk powder had been added.

Relative sizes of typical crystals found in these ice creams are shown (Figure 1). The ice cream seeded with small alpha hydrate crystals developed great numbers of small, almost needle-shaped crystals; whereas, the ice cream containing the milk powder developed parallelogram- or diamond-shaped crystals. The latter ice cream contained fewer crystals, but they were larger and more uniform in size. Neither ice cream became sandy during the storage period. The control sample, however, developed typical sandiness, owing to large clumps of lactose crystals.

It is recognized that lactose in milk powder is present as a noncrystalline glass, containing an equilibrium mixture of the alpha and beta forms *(7)*.



A B C

FIG. 1. Typical lactose crystals found in the ice creams used in this study. Magnification 100 ×. A. Normal ice cream. B. Seeded ice cream. C. Ice cream containing milk powder.

Therefore, an explanation was needed for the presence of crystals in ice cream to which milk powder was added. Experiments were performed to demonstrate the development of crystal nuclei when milk powder was exposed to saturated or slightly supersaturated lactose solutions.

Experiments with saturated lactose solutions and milk powders show that lactose glass is not stable when in contact with a saturated lactose solution. Dry milk powder was sealed in dialyzing tubing and immersed in solutions saturated with lactose at 0, 10, 20, 35, and 45° C. After storage overnight at the respective temperatures, the milk powder was examined under the microscope with polarized light. The powder was a mass of lactose crystals, in each case. Certain supersaturated solutions also produced mass crystallization of the lactose in milk powder, when stored under these same conditions. For example, solutions were saturated with respect to lactose at 10 and 20° C. and cooled to 0° C., before immersing the dialyzing tubing containing the dry milk powder. Examination of the powder after storage overnight revealed large masses of lactose crystals.

That crystallization of the lactose in milk powder also would take place under the conditions found in ice cream was proved by inserting tubes containing dry milk into soft ice cream (23° F.) and into hardened ice cream (2° F.). In both cases, examination of the powder on the day following revealed that moisture had been absorbed and great numbers of small crystals had been formed. This explains the source of the lactose crystal nuclei found in ice cream containing the milk powder—these crystals act as seed to prevent sandiness.

DISCUSSION

In normal ice cream, lactose crystallization is relatively slow, but only a small quantity of crystalline lactose present in a relatively few crystals is sufficient to produce sandiness *(5)*. Seeding the ice cream with crystal nuclei causes rapid crystallization of a large proportion of the lactose. It causes the super-

saturated lactose to be deposited as small, insensible crystals rather than large, slow-dissolving ones; therefore, seeding prevents sandiness.

It was also found that ice cream to which milk powder had been added during freezing did not go sandy *(6)*. Explanation for this phenomenon has been that the lactose in the powder does not go into solution, because the partially frozen mix is essentially saturated with lactose when the powder is added. If this explanation were valid, polarimetric readings made on this ice cream should be the same as in normal, low-solids ice cream, but data show this explanation is not valid. Polarimetric readings prove that lactose crystallizes in ice cream containing milk powder at approximately the same rate as in seeded ice cream. Also, the same quantity of lactose crystallizes in these two types of ice cream, showing that the lactose in the milk powder can not be considered as isolated from that in solution.

Lactose crystallization in ice cream is slow unless crystal nuclei are present. Since crystallization is rapid in ice cream containing milk powder, nuclei must be produced more rapidly than in normal ice cream. The source of these nuclei is the milk powder. This can be shown by submerging milk powder sealed in dialyzing tubes into saturated or supersaturated lactose solutions or into ice cream. After a short storage period, the powder contains great numbers of small alpha lactose crystals.

The fact is that milk powder does not go completely into solution. Individual milk particles maintain their identity and can be observed under the microscope. But this is not the reason why such ice cream does not become sandy. The role of milk powder in preventing sandiness lies in the fact that it causes numerous crystal nuclei to develop. These seed the ice cream efficiently, so that the supersaturated lactose is deposited as minute crystals that are not detectable in the mouth.

SUMMARY

Polarimetric data are presented to show that lactose crystallization proceeds at essentially the same rate in ice cream to which milk powder has been added as in seeded ice cream. The lactose glass of the powder produces a multitude of crystal nuclei when introduced into partially frozen mix, in spite of the fact that the mix may be saturated with respect to lactose. These nuclei serve as seed; consequently, the supersaturated lactose is deposited as small undetectable crystals.

The generally accepted explanation of the role of milk powder in preventing sandiness was shown to be false.

ACKNOWLEDGMENT

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DISPLACEMENT SEPARATION OF SOME OF THE COMPONENT FATTY ACIDS OF MILK FAT [1, 2]

S. KURAMOTO [3] AND J. J. JEZESKI
Department of Dairy Husbandry, University of Minnesota, St. Paul
AND
R. T. HOLMAN
The Hormel Institute, and Department of Physiological Chemistry, University of Minnesota, Austin

Nine fatty acids of milk fat, from butyric to stearic, were separated by displacement chromatography. This method appears to be adaptable to the separation of volatile and nonvolatile fatty acids by first saponifying the fat and then acidifying the soaps on the column just prior to carrier displacement analysis. *Editor.*

Displacement separation is a special technique of chromatographic separation, in which the column is developed by a solution of a substance which is more strongly adsorbed than the components of a sample. After the column is washed with solvent, the sample to be separated is pressed into the column. A solution of the displacer substance is then pressed onto the column and, because it is more strongly adsorbed than are the components of the sample, these are displaced and migrate down the column according to their relative adsorbabilities, the least adsorbed material migrating first. Each substance in the chromatogram thus displaces the component with the next lower adsorbability, and the components migrate in zones one after the other. The zones are detected and the concentrations of solute in the effluent may be measured interferometrically. Acids, so separated, may be titrated with alkali.

Carrier displacement chromatography makes use of relatively large quantities of substances whose adsorption isotherms lie between those of the components of the sample. When the sample components and the added carrier substances are displaced, the constituents arrange themselves in order of adsorbability, and the components of the sample are sandwiched between much larger zones of the added substances. Thus, small quantities of sample constituents travel at the

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[3] Present address, Land O' Lakes Creameries, Inc., Minneapolis 13, Minn.

fronts of the zones of the more abundant "carriers" and, under ideal conditions, are completely separated from each other.

Displacement separation has been used for the separation of fatty acids *(2, 10, 11)*, analogs of fatty acids *(8)*, amino acids *(14)*, sugars *(13, 15)*, glycerides *(5)*, and sterols *(4)*, but its application to the separation of the fatty acids from natural sources has not been extensive. Very useful information on the displacement separation of the fatty acids from C_1 to C_{22} is available *(11)*. Carrier displacement separation of lauric, myristic, palmitic, and stearic acids also has been performed *(7)*. This suggested that the carrier displacement separation of the fatty acids of milk fat could be effected. In this study, displacement analysis of milk fat acids was attempted. However, best results were obtained by the carrier displacement technique.

EXPERIMENTAL PROCEDURE

The apparatus used in these experiments was a modification of the Tiselius-Claesson interferometric adsorption analysis apparatus *(12)*. Hamilton *(3)* made improvements to permit the use of nonpolar solvents. All experiments were performed with the Hagdahl *(1)* coupled filters, and interferometric readings were made at 37° C. in a 160-mm. cuvette. The adsorbent used throughout these studies consisted of one part Darco G-60 charcoal mixed with two parts of Hyflo-Supercel. Packing the column and making observations with the instrument have been described in detail elsewhere *(12)*.

The procedure adopted for the analysis of milk fat was as follows:

(1) *Preparation of the sample:* 25 g. of milk fat was saponified with 17.5 g. of KOH in 125 ml. of 95% ethanol. After refluxing for three hours, the ethanol was distilled off and the fatty soaps were taken up to exactly 200 ml. with water. A 1-ml. aliquot of this soap solution represented 125 mg. of the original milk fat.

(2) *Analysis for butyric acid:* 1 ml. (125 mg.) of the saponified milk fat solution was injected into the top of the coupled filter column. The column was constructed by using a 15.7-ml. filter filled with the Hyflo-Supercel as part of the upper column; the rest of the column was loaded with an aqueous suspension of the Darco-G60 Hyflo-Supercel mixture and had a total volume of 19.1 ml. After the sample, 1.5 ml. of 2% H_2SO_4 and 100 mg. of isopropyl alcohol, in that order, were injected. The large driving syringe containing the displacer (1.5% methyl butyrate in water) was placed on the column and the solution was pressed in. Seventy-five 1-ml. fractions were collected and each was titrated with alcoholic KOH to determine the total butyric acid.

(3) *Analysis for caproic acid:* In a similar fashion, 1 ml. (125 mg.) of the soaps was pressed into the column, followed by 1.5 ml. of 2% H_2SO_4. A 24.1-ml. column was packed with the 1:2 Darco G-60 Hyflo-Supercel mixture with 40% ethanol as the solvent. The additional top filter contained 15.7 ml. of Hyflo-Supercel. The driving syringe filled with 1.2% methyl caproate in 40% ethanol was connected to the column and the solution was pressed in. Seventy 1-ml.

fractions of the effluent were collected, and the caproic acid was measured by titration with alcoholic KOH.

(4) *Analysis for caprylic and capric acids:* 1 ml. of the milk fat soaps was pressed into a coupled filter column having a total volume of 29.1 ml. The top of the column had a 15.7-ml. coupled filter filled with Hyflo-Supercel. The solvent was 60% ethanol. Following the sample, 1.5 ml. of 2% H_2SO_4 and 150.4 mg. of methyl caprylate dissolved in 1 ml. of 60% ethanol were pressed in. The piston-driven syringe filled with 1.5% methyl caprate in 60% ethanol was then connected to the column. Ninety-five 1-ml. fractions were collected and caprylic and capric acids were measured by titration with alcoholic KOH.

(5) *Analysis for lauric, myristic, palmitic, and stearic acids:* In a manner similar to that described for the analysis of the other fatty acids, 1 ml. of the saponified milk fat solution was injected into a coupled filter column having a capacity of 59.8 ml. An additional 31-ml. filter was attached to the top of the column and was filled with Hyflo-Supercel. Next, 1.5 ml. of 2% H_2SO_4 followed by the carriers (47.8 mg. methyl laurate, 98.1 mg. methyl myristate, 122 mg. methyl palmitate) dissolved in 3 ml. of 95% ethanol was pressed in. The driving syringe filled with 1% methyl stearate in 95% ethanol was connected and the solution was pressed into the column. One-ml. fractions of the percolate, totaling 185 ml., were collected and measured for lauric, myristic, palmitic, and stearic acids by titration with alcoholic KOH.

Methyl butyrate, caproate, caprylate, caprate, laurate, myristate, palmitate, and stearate, which were used for carriers and displacers, and butyric, caproic, and caprylic acids were obtained from Distillation Products Industries, Eastman Organic Chemicals Department.

RESULTS AND DISCUSSION

During the earlier stages of this investigation, it was believed that simple displacement analysis could be used for the complete separation of the fatty acids of milk fat. Results obtained by Holman and Hagdahl on saturated acids *(10, 11)* suggested that their systems could be used directly for measurement of butter oil composition. However, adequate qualitative and quantitative separation could not be obtained by simple displacement, because of the overlapping of the narrow zones of the acids. Furthermore, small quantities of the acids were not distinguishable, and a procedure which would clearly separate small amounts of the acid homologs was desirable.

Carrier displacement separation of lauric, myristic, palmitic, and stearic acids was performed by Holman *(7)*, using the corresponding methyl esters as the carrier system. This procedure had the principal advantages of widely separating the homologs and allowing the measurement of small quantities of acids (5 to 10 mg.).

In order to separate the fatty acids of milk fat by carrier displacement analysis, carrier systems were devised to permit the separation of butyric, caproic, caprylic, and capric acids. Carrier separation of lauric, myristic, palmitic, and

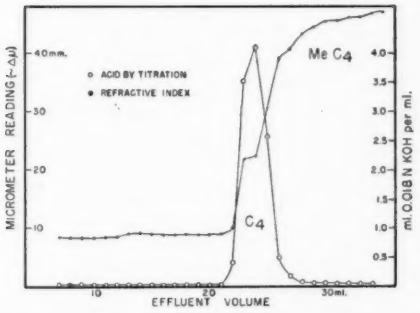

FIG. 1. Single displacement separation of 15 mg. of butyric acid in water on a 9.1-ml. coupled filter column, using 1.5% methyl butyrate as displacer.

stearic acids was performed in 95% ethanol with 1% methyl stearate as the displacing solution *(7)*, but new solvent and displacer systems for the separation of the lower acids had to be devised.

The following conditions were found to allow the separation of butyric, caproic, caprylic, and capric acids: *(a)* butyric acid was separated and measured in water with 1.5% methyl butyrate as the displacer, *(b)* caproic acid was separated in 40% ethanol with 1.2% methyl caproate as the displacer, and *(c)* caprylic and capric acids were separated in 60% ethanol with 1.5% methyl caprate as the displacer. Figures 1, 2, and 3 graphically illustrate typical model separations of these four acids with the solvent and displacer systems described. Several attempts were made to separate these four acids in a single displacement setup, but the wide range of the solubilities and adsorbabilities of the acids and esters does not permit adequate resolution in a single system.

Recoveries of pure butyric, caproic, caprylic, and capric acids added directly to the column were determined by using the chosen solvent and displacer conditions. (See Table 1.)

The recovery of these four acids was almost quantitative in the carrier displacement systems devised.

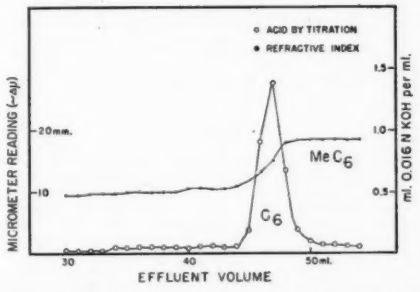

FIG. 2. Single displacement separation of 11 mg. caproic acid in 40% ethanol on a 14.1-ml. coupled filter column, using 1.2% methyl caproate as displacer.

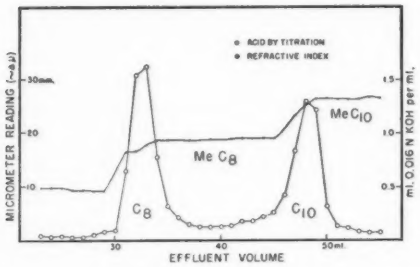

FIG. 3. Carrier displacement separation of 14.8 mg. caprylic acid, 16.1 mg. capric acid, 133.7 mg. methyl caprylate in 60% ethanol on 19.1-ml. coupled filter column, using 1.5% methyl caprate as displacer.

Initial attempts were made to separate the fatty acids from saponified milk fat into steam-volatile and nonvolatile fractions. The fatty acids in the volatile fraction were stored as soaps, and acidified and extracted with ethanol just prior to being subjected to carrier separation. Even so, the obvious losses of these volatile lower acids during handling made it necessary to abandon this procedure. Direct acidification of fatty soaps with H_2SO_4 on the adsorbing column, followed by carrier displacement separation, seemed to eliminate losses through evaporation. The addition of a suitable quantity of the saponified milk fat solution, followed by acidification directly on the column, also provided a rapid procedure for the analysis of the sample, without the need of previous extraction and distillation procedures.

Direct acidification of the soaps on the column resulted in the precipitation of the solid fatty acids, which would not allow adequate percolation of the solvent; but the inclusion of the filter aid (Hyflo-Supercel) as part of the upper segment of the column permitted free passage of solvent. The H_2SO_4 did not complicate the separation, because the excess acid was eluted at an effluent volume equal to the size of the column. Because of the rapidity and simplicity of this process, experiments involving the higher acids were also conducted with it.

Figure 4 illustrates the single displacement separation of butyric acid from saponified milk fat. In this separation, isopropanol, when added with the sample, was found to prevent excessive "tailing" of the eluted H_2SO_4. It is evident that selective separation of butyric acid resulted under the solvent and displacer

TABLE 1

The recovery of pure butyric, caproic, caprylic, and capric acids by carrier displacement separation

Acid	Acid added	Acid recovered	Recovery
	(mg.)	*(mg.)*	*(%)*
Butyric	11.5	11.3	98.2
Caproic	12.6	12.2	96.8
Caprylic	12.3	12.2	99.2
Capric	11.2	11.7	104.0

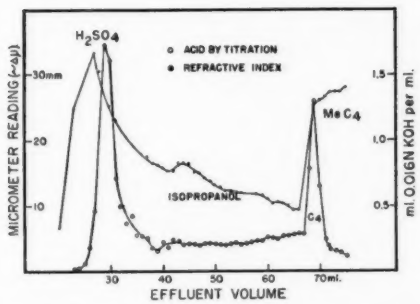

FIG. 4. Single displacement separation of butyric acid from milk fat. Sample — 1 ml. milk fat soaps (125 mg. fat), 1.5 ml. 2.0% H_2SO_4, 100 mg. isopropanol. Solvent, water. Filter, 15.7 ml. Hyflo-Supercel, 19.1 mg. adsorbent. Displacer, 1.5% methyl butyrate.

conditions used. The rest of the component milk fat acids were retained on the column and remained adsorbed in preference to the displacer.

Figure 5 similarly shows the single displacement separation of caproic acid from saponified milk fat. Butyric acid was found to be eluted along with the H_2SO_4, and these two constituents emerged as an elution spike.

The carrier displacement separation of caprylic and capric acids is shown (Figure 6). Methyl caprylate was added with the sample as a carrier for caprylic acid and further served as a "wedge" between caprylic and capric acids. The spike which occurred at an effluent volume equal to the size of the column, consisting of H_2SO_4, butyric, and caproic acids, is not shown.

The carrier separation of lauric, myristic, palmitic, and stearic acids is shown (Figure 7). The carriers used for this separation consisted of methyl laurate, methyl myristate, and methyl palmitate. Under the conditions of development, acids with chain lengths less than 10 carbon atoms were not adsorbed and were eluted along with the H_2SO_4. On the other hand, the other fatty constituents, notably the nonsaponifiable materials, were more strongly adsorbed than the

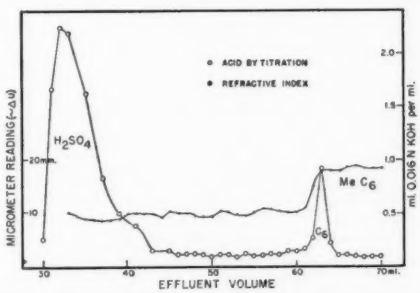

FIG. 5. Single displacement separation of caproic acid from milk fat. Sample — 1 ml. milk fat soaps (125 mg. fat), 1.5 ml. 2.0% H_2SO_4. Solvent, 40% ethanol. Filter, 15.7 ml. Hyflo-Supercel, 24.1 ml. adsorbent. Displacer, 1.2% methyl caproate.

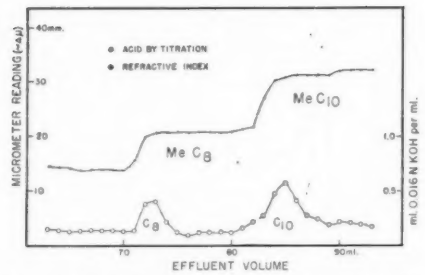

FIG. 6. Carrier displacement separation of caprylic and caprie acids from milk fat. Sample — 1 ml. milk fat soaps (125 mg. fat), 1.5 ml. 2.0% H_2SO_4, 150.4 mg. methyl caprylate. Solvent, 60% ethanol. Filter, 15.7 ml. Hyflo-Supercel, 29.1 ml. adsorbent. Displacer, 1.5% methyl caprate.

displacer solution. As a consequence, a selective separation of laurie, myristic, palmitic, and stearic acids was accomplished by proper solvent and displacer selection.

The length of the methyl myristate step and the high titration values obtained for effluent volume fractions 145 to 155 ml. indicated that an acid having adsorptive characteristics similar to methyl myristate was being displaced between myristic and palmitic acids. Holman *(9)*, investigating the adsorbability of oleic acid with reference to a carrier system consisting of methyl laurate, methyl myristate, and methyl palmitate, has shown that oleic acid is separated within the methyl myristate zone. From this information, it is safe to assume that oleic acid, present in milk fat at a concentration of 25 to 30% (by weight), lies in this zone.

There is rather good agreement between the values reported for milk fat acids by Hilditch *(6)* and those found by displacement separation.

Only with palmitic and myristic acids do the weight percentages vary to any extent. The values given for myristic and palmitic acids include oleic acid, which

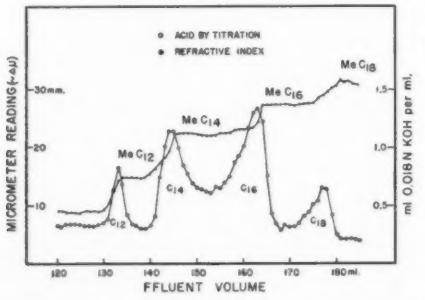

FIG. 7. Carrier displacement separation of laurie, myristic, palmitic, and stearic acids from milk fat. Sample — 1 ml. milk fat soaps (125 mg. fat), 1.5 ml. 2.0% H_2SO_4. Carriers, 47.8 mg. methyl laurate, 98.1 mg. methyl myristate, 122 mg. methyl palmitate. Solvent, 95% ethanol. Filter, 31 ml. Hyflo-Supercel, 59.8 ml. adsorbent. Displacer, 1% methyl stearate.

TABLE 2

Comparison of the weight percentages of milk fat acids obtained by carrier displacement separation and methyl ester distillation

Fatty acid	Carrier displacement	Methyl ester distillation (Hilditch)
		(range)
Butyric	3.1	3.0 — 3.7
Caproic	1.8	1.3 — 2.0
Caprylic	1.4	0.5 — 1.7
Capric	4.1	1.9 — 3.8
Lauric	5.3	2.8 — 7.3
Myristic	20.9	8.1 — 17.1
Palmitic	31.9	25.3 — 29.0
Stearic	12.0	4.8 — 11.2

occurs throughout the methyl myristate zone and, therefore, is not adequately separated from either myristic or palmitic acid.

It thus becomes evident that direct acidification of a saponified milk fat sample followed by carrier displacement is a relatively rapid procedure for the estimation of the more common aliphatic acids of milk fat. Instead of resorting to an initial group separation involving extraction and distillation procedures, selective separation may be accomplished by the proper choice of solvents and displacer solutions. Conversion of the fatty soaps to acids with sulfuric acid did not complicate the chromatogram, because the excess acid was not adsorbed and was found to be eluted at an effluent volume equal to the size of the column. This, however, makes it difficult to identify fatty acids of less than four carbons in chain length.

By using the four chromatographic systems, the common saturated fatty acids of milk fat were measured with good recovery. The nonsaponifiable fractions, notably the vitamin A, carotenoids, and sterols, are probably retained by the adsorbent; that is, they are not displaced by 1% methyl stearate. The identification of polyethenoid acids was not attempted, because of their relatively small quantity in milk fat. Holman *(9)* observed that unsaturation decreases adsorption; that is, one isolated double bond decreases adsorbability equivalent to shortening the chain length by two carbon atoms; and he found that oleic acid followed this rule, being separated throughout the methyl myristate zone. Thus, monoethenoid acids, along with linoleic, linolenic, and other trace fatty acids recently reported in the literature, were not distinguished and in all probability are included in the values for the saturated acids. Although no provisions were made for their detection, identification could be made by the application of proper assay techniques on the appropriate effluent fractions.

SUMMARY

With the technique of carrier displacement chromatography, the separation of some of the fatty acids of milk fat has been shown to be possible. Nine fatty acids, from butyric to stearic acids, have been separated by the proper choice of

solvents and displacer concentrations. Losses of the shorter chain acids through volatilization could be overcome by introducing the sample as a soap, followed by acidification directly on the column.

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

New Packaging Methods and Their Value[1]

R. E. ELDRED

National Butter and Cheese Department, The Great Atlantic and Pacific Tea Co. Chicago, Illinois

The subject "New Packaging Methods and Their Value" is one of such latitude that it is difficult to know exactly where to start and where to stop. Some of the packages in today's market were introduced 25 to 30 years ago and are still highly rated. Since then, many have appeared and disappeared, with new ones entering the market each day. In fact, we have been advised that some 200 store items are offered to the average grocery chain each week, the majority of which are in packaged form. What may be old to some observers may be comparatively new to others. Therefore, it would seem logical that we review briefly certain developments of merchandising and packaging, of both butter and cheese, so that we may have a common background for discussion of their value and may consider the packaging future.

Before introduction of self-service in grocery stores, butter was a regular item on the shopper's list. The customer's butter order was filled by cutting and weighing the product from a crock or wooden tub. The wrapper used was often manila paper and, later, parchment or waxed paper. In larger stores, when customer traffic was heavy, butter was cut and wrapped a short while before sale, thus originating the so-called "tub-cut" butter which continues in existence in some markets today. Size of the package would vary from one to three pounds, depending upon customer demand. Later, various simple type butter-cutters, of which the Friday cutter was very popular, were used in the creameries to precut for hand-wrapping, prior to delivery to consumer outlets. The one-pound solid was the standard package. Then we observed mechanical equipment for cutting bulk butter and extruding it through an orifice from which uniform pieces could be obtained by use of wire-cutters. This accelerated the packaging operation, as it permitted employing girls to handle the wrapping process. This was the forerunner of the modern butter print room which came into being during the thirties. Today, the print rooms have mechanical equipment for cutting, printing, wrapping, and cartoning with a minimum of labor. Automatic mechanical equipment for special type packages is also found in the present-day butter print rooms. Not only do we observe production of one-pound solids but half-pound prints, quarter-pound prints, half-pound patty rolls, individual serving patties, and butter slices. The continuous butter-manufacturing operations also have equipment for packaging direct. Such mechanical equipment has enabled production of packaged butter in larger quantities and with a reduction in cost.

Of course, the demand for this type butter on a large scale was created by introduction of the self-service food markets, where prepackaged butter was necessary. The refrigerated self-service dairy case gave customers access to these products without assistance from a sales clerk. This introduced problems of making available packaged butter with maximum freshness, standardized uniform quality, proper weight, and attractive appearance—all at minimum cost. (The ideal butter package should furnish protection from absorbable odors; from shrinkage, staleness, and oxidation; from deformation under normal handling conditions, and from contact contamination.) Butter which could be printed and wrapped hundreds of miles from point-of-sale was required to sell itself in the self-service dairy cases.

During the past two decades we have observed progress in the development of butter wrappers of which plain parchment, treated parchment, heat-sealed paraffin paper, and aluminum foil either with or without parchment lamination have predominated. The cartons have consisted of wax-dipped Elgin, Western (California), and Eastern styles. Butter has been packed into wax- or paraffin-coated fiber cups or rolls, to produce the effect of the crock or farm print butter of earlier days. Glass jars are frequently used, particularly for sweet and whipped butter. Small amounts of canned butter, for long keeping, can be found in some markets. Several innovations in butter packages have been introduced but have not re-

[1] Presented at the 24th Annual Dairy Technology Conference, The Ohio State University, February 5, 1957.

ceived enough acceptance to warrant their continued existence. One of these was a half-pound butter mold which utilized a formed vinyl plastic cup slung in a paperboard collar. The flexibility of the molded sheet permitted it to be turned inside out for removal of the butter, without damage to the mold and without finger contact. After use, the mold and collar could be pressed back on, for storing in the refrigerator.

Basically, one can find in consumer channels today the very same packages as just mentioned. Outlets close to production and packaging sources permit certain packages which cannot be used elsewhere. A packer can use a single parchment wrapper where shrinkage, handling, and flavor protection factors can be minimized. Double parchment-wrapped butter and single parchment wrapper with heat-sealing overwrapper and foil wrappers have assisted in prevention of shrinkage losses. Wrapping butter in parchment and putting it in paraffined cartons has afforded protection against both shrinkage and handling. Use of aluminum foil was introduced some years ago for the primary purpose of increasing longevity of freshness and of reducing shrinkage, two very vital factors in butter merchandising. We observe it primarily on quarter-pound quarters of top-quality sweet-cream butter, sweet (unsalted) butter, and whipped butter. It also has proved beneficial in that it dresses up the package (variable colors and designs can be advantageously applied). Of course, full merit is not gained unless it is used in an open- or window-faced carton. Although foil is being increasingly used, its cost in many instances prevents more widespread usage. Further, there are variable opinions as to its protective properties after the butter has been packaged for a certain number of days.

The heat-sealed overwrap, for which a machine was introduced a few years ago in the East, has proved successful where distribution over great distances is not a factor. Its merit rests in reducing shrinkage and lowering cost. However, its primary limitation is that it does not afford much security in handling. This overwrap is primarily for one- and half-pound prints. Its use seems to have leveled off in recent years.

Industrywise, the paraffined carton is still considered the package for providing the most desirable characteristics essential to successful merchandising. Style of carton is a factor in several sections of the country; the so-called Elgin style is the most popular. However, the use of the Eastern (square) package is slowly increasing. It affords a greater surface on one panel, whereon advertising and design can be more effectively displayed. It has proved effective on foil-wrapped quarters which can be shown through an open face on the package. The margarine industry has used the Eastern-style carton more effectively than has the butter industry. Of course, cost is a factor of consideration when contemplating its use. The Western (California)-style carton, as the name implies, is found primarily in the western section of the country. Elsewhere, it is used by dairies or milk plants which distribute butter on home-delivery routes.

The use of individual-serving butter patties for restaurant, cafeteria, hotel, and institutional requirements is definitely on the increase. Their saving, in labor, their sanitary serving and uniformity of size aspects seem to justify the added cost involved.

As previously mentioned, so-called "tub-cut" butter is still marketed in some areas to a limited extent. Perhaps more is found in the southern section of the country than elsewhere. It is claimed that many customers find great satisfaction in exercising freedom in selecting just the right size package of butter. Further, a jumbled mass display of store-wrapped butter provides effective merchandising. Of course, the 63- and 32-lb. wooden tubs have given way primarily to the 60-lb. fiber box, for warehouse assembling from creameries.

The half-pound round print patty, either parchment- or foil-wrapped, is marketed primarily below the Mason-Dixon Line. Size of package is such as to permit serving on the table in similar manner to tub-cut or old-fashioned crock butter.

The sale of whipped butter has, in recent years, received considerable attention. Whipped butter possesses an increased volume without any effect on the weight; thus, the size of package denotes a psychologically better buy. Thus, deception satisfies a customer's ego to the point where this item is considered as added trade by the retailer. There is claim that whipped butter possesses certain spreading qualities not afforded by regular butter. It can be found on the market in quarter, half, three-quarter, and one pound prints, as well as in six- and eight-ounce cups and jars.

The present-day diet-conscious customer has pushed the sale of so-called sweet (unsalted) butter to the point where most stores stock it regularly. Formerly, this item was confined to the larger metropolitan markets, where specialized trade was available. In addition, technologists have developed a sweet butter of high quality, with increased longevity; thus permitting distribution great distances from source without fear of deterioration. The consumer's taste is being cultivated; many hotels, restaurants, and dining-cars serve sweet butter exclusively. The trend indicates increased usage of unsalted butter. This item, with its high degree of perishability, commands the best protective packaging available.

Butter slices were first introduced in the New

York market several years ago and have since been produced in other areas by two or three concerns. Half-pound packages containing two one-quarter pound prints (each print containing 16 equal portions) were thought to create consumer acceptance, but the reaction has been slow.

So much for the past and present status of butter. Let us focus attention on cheese for a few moments. Cheese sold in service stores was cut from traditional styles such as Longhorns, Daisies, Flats, and Midgets, weighed and wrapped according to each customer's desire. The cheese bell, which covered a cheese used for store-cutting, may be a memory to some of you. The clerk would often pride himself in being able to cut a wedge of cheese to the exact weight desired. Manila, parchment, and waxed papers were used at various times for wrapping the cheese. The introduction of process cheese in the early twenties had perhaps the largest impact upon cheese packaging and merchandising of any new development in the cheese industry. Process cheese permitted distribution of a product with keepability, in consumer-size loaves. This doubled American consumption of cheese (when compared to amount consumed during the previous three decades). The five-pound loaf was first introduced, followed by smaller packs. The wooden box for the five- and two-pound loaves continued to the early forties, when it gave way to the fiberboard box. In some markets, process cheese supplanted to an extent the over-counter sales of custom-cut natural cheese. It was a boon to the cheese industry. However, packaging did not show much progress until the introduction of self-service dairy departments in supermarkets.

Bulk natural cheese from the various traditional styles was cut and wrapped in the store prior to placing in the refrigerated self-service dairy case. Cheddar, Brick, and Swiss contributed most of the cheese distribution. However, shortly thereafter, other varieties gained attention, such as the Gouda, Edam, Muenster, Blue, Brie, Gold N'Rich, and Limburger. Later, the Italian varieties and other cheeses received recognition. This transformation took place largely in the thirties. The distribution of prepackaged process cheese foods in addition to process American was well under way just prior to the outbreak of World War II, as was the use of 20-lb. rindless Cheddar blocks for cutting and wrapping into one-half- and one-pound consumer-size packages, in areas away from the stores. During this same period cheese cut in stores was wrapped in waxed paper, parchment, cellophane, or pliofilm, whichever was available.

Following termination of the war, expansion of supermarkets with self-service dairy cases resulted in creating demand for consumer-size cheese packages which were weighed, labeled, and price-marked for customer selection. The products had to do their own selling. Introduction of prepackaged process cheese slices in 1950 was another big step toward large-scale cheese packaging away from the stores. Since that time, there has been the development of prepackaged natural slices of Cheddar, Swiss, Brick, Muenster, Colby, Monterey, Mozzarella, Provolone, and Caraway Cheese. We have also observed the introduction of prepackaged Blue, Gorgonzola, Port du Salut, Gold N'Rich, Provolone, Romano, and Parmesan, just to name a few. Pasteurized cheese spreads, either in loaf form, cups, or jars, have commanded much attention. Owing to the emphasis on prepackaged cheese, there developed widespread production of Cheddar, Swiss, Brick, Muenster, Port du Salut, Provolone, etc., in rindless block form. As would be expected, due to the variability of cheeses, the problems of mechanical cutting and wrapping of cheese have been many-fold. The wrapping materials have been, and are, numerous—some of which are cellophane, Pliofilm, Parakote, waxes, and plastic coatings, polyethylene, acetate, Cryovac, Flexvac, foil, Saran, and Mylar. In addition, process items are packed in glass, plastic, tin, cardboard, and foil containers. The shapes, sizes, and colors of prepackaged cheese items have been, and are, most variable.

Why is packaging receiving much more emphasis than ever before? We believe some of the reasons are: (1) the largest percentage of food is sold in self-service stores; (2) there is a growing demand for packages which will assist in keeping the dairy cases stocked with a minimum of labor and cost of labor. (It would be most difficult, if not impossible in some markets, to store-cut, weigh, wrap and price-mark cheese in sufficient quantities to cover the peak demand periods of the week's total business.); (3) sanitary and health regulations are more numerous and demanding; (4) improved methods of transportation permit distribution of packaged merchandise without fear of spoilage; (5) the development of newspaper, magazine, radio, and television advertising and promotion has created consumer demand for brand-name products of standardized and uniform quality; (6) our inflationary economy has been such as to support the costs of packaging, advertising, and promotion; (7) prepackaged cheese permits distribution into retail outlets which were not equipped to handle store-cut and wrapped items, (8) packages have been found effectively to convey information direct to the consumer via printed matter and designs on the wrappers. This information may be recipes, suggestions for usages, methods of handling and of care in the home, historical notes, related food usages, etc. This consumer contact has been found to enhance demand for the product.

Thus, one can readily recognize the responsibility of a package. As far as the butter industry is concerned there has been, until recently, somewhat of a state of lethargy in making the most of the package. Of course, progress in printing, color offset, lithography, and design has come into its own only in recent years. Frankly, it has been only in recent years that the butter industry has recognized that its product could not be taken for granted as far as consumer acceptance was concerned. Butter was the spread on most tables, and thus a standard item on the average grocery shopping list. A combination of factors has been responsible for butter being toppled from a so-called "pedestal"; namely, legislative protection, price (due to rising economy), research and development of other spreads, reports on correlation between fat intake and heart diseases, and the lack of proper margins for advertising and promotion. Historically, butter has been a low-margin product. Only the bare costs of marketing were removed from the returns to the creameries. Manufacturers of other spreads have allowed for vast merchandising programs and have taken a decided lead over butter in its effort to reach the consuming public. Fortunately, the butter industry is recognizing the necessity for expending funds for keeping its product in competition. The American Dairy Association has spearheaded a program of improved butter package design, advertising, and promotion. We note creameries, butter receivers, and retail organizations supporting this program. Pictorial designs in multiple colors are quite prevalent; recipes are printed on the cartons; special holidays are being recognized for butter merchandising. These have assisted in shaking the industry from its status quo position. Of course, the trial-and-error period is still much in effect. Many feel that carton dressing is a lasting and positive solution, but it must be recognized that costs and inventory control of carton stocks are factors of consideration. The present status indicates there is a happy medium which has yet to be determined.

Another observation is that, historically, butter has been included with flour, sugar, and coffee as being the forerunner of grocery merchandising. They were all volume items in the low gross-profit classification. Customers have been aware of this, based upon their quick reaction to price fluctuations. Butter was expected to remain in this category; hence, it has been most difficult to shake this premise. Even with the present price structure of butter, customers closely watch its retail, and respond accordingly. Those some people who critically study the food ads and prices will enter other type merchandise stores with only small feeling toward the often high corresponding cost of the items. This is cited merely to show that butter has one burden from which it must shake itself loose, if it is to enter the merchandising field on a truly competitive basis. To be sure, progress is being made, but it is slow.

It must also be recognized that, in general, these spearheading grocery commodities, as mentioned above, do not possess the abundance of merchandising glamour so essential to the modernized version of advertising and promotion. These products possess value essential to one's well-being, but the characteristics have been too intangible for merchandising standards.

Now, spotlighting cheese, we find it possesses perhaps the greatest number of desirable merchandising characteristics of any food item. It has variety of color, texture, flavor, shape, size, usage, and keepability. It is available in either the natural or processed state. Its historical development is variable, and widespread in many countries over many centuries. Its mention is abundant in folklore. Thus, the modern self-service dairy case has access to colored or white Cheddar wedges or cubes of variable flavors; red, oval Goudas; wedges of blue mold–veined blue cheese and green mold–veined Gorgonzola; yellow waxed cubes of Kuminost; brown cubes of Primost; packages of both sliced and chunk Swiss, Brick, and Muenster; Process Cheese slices of regular American, sharp American, Swiss, Pimiento, and Brick flavors; cheese foods and cheese spreads in many sizes and shapes of containers, and the Italian varieties of Provolone, Mozzarella, Romano, Parmesan, Salamini, Boccini, Seamorze —just to name a few. Excepting certain processed items, grated cheeses, and process Gruyère, the above-mentioned cheese items, and many more, are in competition in the refrigerated dairy case—not only with each other but also for the space required by other refrigerated items such as butter, margarine, milk, cream, cottage cheese, eggs, biscuits, horseradish, canned fresh fruit, pancake batter, gelatin salads, yeast, orange juice, ice-box cookie dough, etc. The modern food store operator, handling well over 3,000 items, will devote time and space only to those which receive regular customer response. Butter with a turnover of 77 times, and cheese with some 35 to 40 times, per year, have given good accounts of themselves. However, a continuous problem pertains to the number of brands of butter and the number of varieties of cheese necessary to permit greatest efficiency of the dairy case.

As this competitive situation brings packaging into full focus, let us review the basic requirements of a successful package: (1) It must command attention—its shape, colors, size, and design are factors of consideration. (2) It must sell itself by having "eye appeal," thus creating a "buy-me" atmosphere. (3) It must draw attention to and furnish protection for

the product. For repeat sales, the product when it reaches the consumer's kitchen or table must not deviate too much in flavor and quality from the time it was packed. (4) It must clearly state the contents, the legal name of the product, the brand name, if any, the weight and ingredient clauses as may be required. The package should easily convey to the customer the "what" and "how much" of its contents. (Time is a factor with most shoppers.) (5) It must convey adequate directions for use. Opportunity is presented for the package to assert itself in a bid for use of its contents in various ways, singly or with related products. (Pictorial designs assist in this endeavor.) (6) It must be attractive when displayed in a mass. Often a package presents a different appearance as a single unit than in a multiple-unit display. It is essential that the package be designed to present its best under any circumstances of display. (7) It must be of the size suited to the majority of homemaker usages. Consumer reasearch is most essential in ascertaining this size. The matter of "left-over" after initial usage of the package must be considered. (8) It must be clearly priced—not only the package itself but also the price per pound—in order to prevent deception, which is so frequently observed. There also should be ample space for price-marking. (9) It must be clearly coded, with date of packing or expiration of sale date, so that proper stock rotation can be applied in the store. Freshness control is most vital to successful merchandising—there has been far too much laxity in attention to coding. In fact, many butter packages on the market today are either without code or, if it is present, it is not clear enough to be of much benefit.

As the modern supermarket is forcing the housewife to eliminate use of a shopping list, it is most essential that its products be at their buying best. (She formulates her menus after she enters the store and observes the offerings.) Cheese and butter will receive their due share of attention if they are merchandised properly. Let us not forget that a package should assist in making a customer want it—not need it, but want it. If shortages of food were to exist in this country the time, effort, and expense of most merchandising would be wasted. The full effect of merchandising exists only when supply exceeds demand—the condition at the present time.

So far we have given emphasis to packages as they affect the customers in the stores, but the receiving warehouse and store operators should not be overlooked. Due to limited space in the dairy case, and the competition for this space, it is essential that only those quantities be delivered which will fit into the space allotted to them. Capital tie-up in inventory is a factor of consideration in this respect. The packer or manufacturer should make available the size units suited to store requirements. We have observed instances where items packed two dozen per case did not receive much warehouse or store personnel attention until they were packed in smaller containers. Even though costs are usually reduced with the larger packs, they are often economically impracticable. Many operators have found it practical to pack six units in a carton, with two or four cartons per case. These cartons should be strong enough to protect the merchandise on its way from warehouse to store. It is common practice to fasten a number of single units into bundles, with strapping to facilitate handling. Each store's requirement can be removed from the bundle in the receiving warehouse.

Each shipping case should be clearly marked with reference to its contents, number of packages, and coding, to permit proper warehouse stock rotation. Codings should be standardized, so that all parties handling the merchandise can make proper interpretations. These markings should appear on more than one side or panel of the case to eliminate extra handling. It is essential that the shipping cases be capable of being easily opened. Tear tapes, perforations, and indentures are in use. However, there is further need for improvement. Another factor often overlooked is that of producing packages which will stack easily when placed on display in the stores, and of producing cases which will stack easily in the warehouses. As mechanized warehouse handling equipment is becoming standardized, it is well to have the cases fit on the pallets and be strong enough to withstand abuse. Also self-service store treatment is much rougher than in a service store. Broken packages are costly to retailers. Whether it be in the warehouse or in the store, those cases or packages which are easiest to identify, easiest to decode, easiest to lift, easiest to open, easiest to price-mark, easiest to stack, etc., will receive priority attention.

We believe the value of packaging in general is fairly self-explanatory. In this period of expanded economy and high rate of employment, the demand has been tremendous for convenience foods and labor-saving devices. It is reported that in 1956, 29% of the nation's married women had jobs outside their homes and it is expected that this will reach 41% by 1975. As the percentage of married women working away from home increases, the demand for "easy meals" will continue. The population growth rate cannot be overlooked. In 1950 the population was 153 million; last year it had increased almost 10%, to 169 million. The projected population figure for 1965 is set at 193 million and for 1975, 228 million. As these people must be fed with minimized efforts, one cannot treat lightly the future of packaging.

Before concluding, it might be well to note a few observations which must be considered as having a bearing upon the future of butter packaging and, more particularly, upon cheese packaging:

(1) Most packaging, as we have observed it, has been expensive, because the value of the finished package must reflect the costs of experimentation, intricate packaging equipment, packaging materials, labor, and overhead items. As long as family incomes accelerate and keep pace with the inflationary trend, customers will undoubtedly support such items, but any decline in over-all incomes would find many packaged items without a market; a customer would soon terminate expenditure of 79¢ per lb. for prepackaged Swiss and would buy 59¢ store-packed Swiss; forty-nine cents per pound Cheddar would also supplant 69¢ prepackaged Cheddar. This could go right down the line; an easier labor market would permit more help for stores and for maintenance of the dairy cases of store-packed merchandise.

(2) Another observation is that much cheese is being manufactured to satisfy the requirements of the packaging operations rather than the consumer. We have seen pasteurized and standardized milk Cheddar of uniform size and shape produced essentially for cutting and wrapping, as the packager is interested in minimizing trim and shrinkage losses and in obtaining packages of standardized weights. There is nothing wrong with this, providing the finished product possesses the flavor and quality desired by the customers, but we regret that too many discrepancies exist. Today unsmoked Provolone, Muenster, Brick, Mozzarella, and Monterey are being manufactured into blocks for slicing operations, and it is frequently most difficult to differentiate these products, let alone associate them with those which were manufactured over the years in their traditional styles. It may be that customers may react unfavorably to this trend. If so, the cheese industry will not be unseathed.

(3) During this period of inflation and rapid development in packaging we have observed a number of processed cheese items which do the industry no credit. The products are technically legal, the packages are beautiful and conform to modern packaging, but the contents do not keep pace. But we believe we observe a tendency towards separating the sheep from the goats, a job which consumers—not regulatory measures—will need to do. Novelty packages will come and go as long as there are plentiful supplies and an economy to support their introduction. We are observing the tendency to decrease the package contents, in order to focus customer-attention on price per package rather than on price per pound. Many shoppers select the items in line with the amount they wish to pay, rather than give emphasis to the economy. While certain merits exist in this trend, we hope for the sake of moving the large production of butter and cheese into domestic consumer channels, and for giving the housewife her maximum money's-worth, that caution will be exercised.

There are ever-changing innovations in packaging. That which is new today may be obsolete tomorrow. We have observed this many times in recent years and can expect more of the same as new ideas are developed. We recently heard this quotation, which we feel is apropos: "Ideals are like the stars. We never reach them, but, like the mariner, we chart our course by them."

Using Plant Personnel Efficiently

CARL W. HALL

Department of Agricultural Engineering, Michigan State University, East Lansing

The cost of processing and bottling milk in 1955 was about 18 cents of the dollar paid by the consumer, of which six cents was for wages and salaries.[1] Many of the other expenses connected with plant operations such as repairs, depreciation, electric power, fuel, water, and other miscellaneous costs are greatly affected by the attitude and actions of the employees. The importance of the plant personnel is considerably more than six cents out of the dollar. In 1954, the net profit after taxes was 2.4 per cent of the sales. By inefficient use of plant personnel, a company could be moved into the loss, instead of the profit, column. For example, if there were three or four extra men in a plant of 40,000-pound-per-day capacity, the operation could show a loss instead of a profit. To remain competitive in an expanding economy, it is absolutely essential that the efficiency of any commercial endeavor be improved. In a dairy plant, this improvement is usually made by maintaining the same number of workers, but at the same time increasing the volume of business—therefore, the net output per man-hour of labor.

Too often we blame the worker for inefficiencies in the plant. Usually, however, the fault of an inefficient operation is the responsibility of management, in that equipment is not properly selected, installed, and maintained to utilize the worker's abilities at peak efficiency. A psy-

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[1] MARKETING COSTS AND MARGINS FOR FRESH MILK, *Misc. Pub. 733*, AMS, USDA, Washington, D. C., October, 1956.

chological barrier can be established between management and workers when workers are blamed for low efficiency.

On the other hand, there are beneficial changes which can be made in the operation which will provide leisure, add to the comfort of the worker, and reduce the hazards of his work. From this standpoint, changes in plant equipment and its arrangement are often necessary or advisable, even though not justified from a purely economical basis. The term "labor saving" has been used considerably, but "labor serving" would be more accurate because, in many cases, improvements can be made which lighten the worker's job and help him to become more satisfied with his task, without necessarily saving time. If time is saved by improving the method, the worker must be gainfully employed at some other assignment to improve plant efficiency.

If plant personnel are used efficiently their job must be; 1) to think, 2) to do useful work.

Each plant operation should be analyzed with a questioning attitude. Encourage and permit plant personnel to make suggestions or changes. They are the best source of time-study ideas. We are in the "do-it-yourself" age and perhaps this attitude can be developed in plant personnel. Resistance to change is overcome if the workers' suggestions are put into practice.

It may be difficult in many instances to determine what is meant by useful work. Perhaps we might explain useful work as that task which a worker can do satisfactorily and more economically than certain equipment can. A girl in a packaging line was shown how a package could be moved from one position to the next by using a circular movement, without lifting and turning, as was done previously. She changed her procedure at the suggestion of the foreman, but about one-half hour later when the foreman came back to check the operation, he saw she was following the old method. Thereupon, he asked why she had changed back; her reply was: "I am getting my exercise." I wonder how many plant operators are paying people to get their exercise.

Plant personnel are not used efficiently unless the equipment is matched to their ability. Here I want to point out a very important fact. It is necessary periodically to revise recommendations on equipment sizes. Labor charges and equipment change rapidly. Only a few years ago, the rotary can-washer was considered the most economical unit for a plant receiving less than 20,000 lb. per day. Now it is not economical for a plant larger than 10,000 lb. per day. The same has happened with high-temperature short-time pasteurizers. Development of cleaning-in-place has changed our whole concept of equipment layout, selection, and operation. The cleaning of heat exchangers, ice cream freezers, and homogenizers until very recently has been considered as a job which could not be carried out by cleaning-in-place.

The following items should be investigated to improve the work method and decrease time requirements.

Relieve hands of work that can be done with the feet. For closing or opening the valve on the weigh-can, a foot-operated valve would relieve the hands for more valuable work. The control at the bottle-filler, which permits the cases of bottles to go into refrigerated storage, should be foot-operated. Jigs for holding equipment which is being washed and sanitized can be operated with a foot-control.

Eliminate reaching. Controls are often located so the worker has to step or walk to reach them from his work position. All tools and equipment should be within easy reach of the operator. The valves at the can-washer should be easily accessible by the man dumping the milk. It is often possible to place an extension on valves, to extend them to the work position. Have washing powders and solutions located near point of use. Having more than one storage for washing materials may be economical. The pencil for recording the weights of the milk should be located where it is easily and quickly accessible. The tool used for loosening the lids of incoming cans should also be within easy reach. The water valve used for cleaning should be close to the point of use. Gauges should be located at a height where they can be easily read by the workers.

Pre-position tools. Having the tools and materials within easy reach of the operator is not enough. A tool should be pre-positioned so that by one grasp of the fingers it is held in the position it will be used in at the job. For doing work with a pencil, the pencil should be pre-positioned so that it is not necessary for the operator to turn it in his hand before writing. The motion preceding the operation in question should end by placing the tools and equipment in the proper position. Place wrenches in a position so that with one grasp and movement they are in the work position. At the end of washing, equipment parts should be in a position from which they can be quickly assembled without loss of motion.

Motions should be balanced and both hands should work simultaneously. This practice is most applicable at the bottle-filler and bottle-washer. Both hands should work together simultaneously in carrying out the same job. Loading and unloading cans, cartons, cases, and material into and out of dry storage provide operations for applying this principle.

The hands should not be used as a holding device. Equipment which is being washed should be held by jigs or fixtures, so that the hands are relieved of the job of holding and can be used for more important tasks. Automatic cleaning devices should have nozzles mounted so

that they will not need to be held by the hands. It should not be necessary to hold cans or bottles for feeding into equipment. Automatic indexing equipment is a must in practically all operations.

Use a drop delivery. Cartons, cases, washing powders, bottles, etc. can be dropped into the processing or adjoining room by means of a chute from a storage above, thus reducing the handling. Gravity can often be utilized to carry out a major part of an operation.

Can we combine operations? The HTST pasteurizer is an example in which heating, pasteurizing, and cooling are carried out in one piece of equipment. Investigate the possibilities of combining hammers, wrenches, screwdrivers, etc. into one tool, where these are used approximately at the same time and place. The pencil is an example of a writing tool and erasing tool combined into one tool.

Can we eliminate operations? This procedure can be applied to many operations and offers the greatest potential for improvement. In many cases little if any financial investment is involved and usually a reduction in plant costs is accomplished. Question the necessity for each operational step. Milk can be separated without the customary heating. Milk bottles can be covered by a hood without a cap. Excessive rinsing during the washing operation often accounts for an excess of 50 per cent in cleaning time. You will find many other operations in your plant which can be eliminated; for instance, it might be an extra copy of the weight slip; it might be carrying sample bottles, etc.

Can we change the sequence of operations? One item of equipment will often limit the output of an operation involving several pieces of equipment. By removing the equipment which is slowing the operation, it may be possible to insert it in the process at another time. The clarifier in the receiving room is usually too small to process the milk as rapidly as it is dumped. Clarifying after storage might be a solution to this problem. Often by changing pumps or providing a surge tank, an even flow of product is assured.

Can we simplify operations? Mechanization isn't the answer to all of our problems. We can often eliminate a machine or select another machine which has a simpler operating technique. The use of high speeds for operating equipment isn't always the answer, because new equipment is usually required for higher speed, and often spillage is created and jamming of the machine occurs more readily unless designed for the high speed.

Procedure

List the jobs which you would want least to do. These are the ones which should be studied first. These jobs would probably include, cleaning of equipment, lifting and carrying of packages and products, and checking by counting and tallying the quantity of product processed or stored.

Time lost due to change-over of equipment for different products or sizes of containers, mechanical maladjustments, hard-to-reach valves and poor balance between adjacent equipment can be located at each operation. Heavy jobs, such as casing and lifting cases, can be done with conveyors, casers, and stackers. These changes provide jobs for workers which are less tedious, with less repetition and eliminating lifting. Automatic recorders and controllers are desirable, not to "check" personnel but to furnish management with information which if not available might cause them to suspect workers rather than "check" equipment faults.

It is important that all changes made in plant operations be approved by management. Improving an operation in the plant whereby the worker is actually idled while being envied by his co-workers may result in unsatisfactory labor conditions. In such cases, changes should be planned but not carried out until an appropriate time designated by management.

In conventional analysis of plant operations, if it is seen that a man is not kept busy at his job, the implication is that he is not doing his share. Many of the evils which have been attributed to time-study and to work improvement could be eliminated if the lack of work by the employee would be attributed to the machine he is attending. It is important to point out that equipment should be designed and arranged to utilize, not to expedite, labor. It is usually necessary to balance the cost of equipment against the value of labor saved. Cost of operation of many plants could be reduced five per cent by adopting more efficient practices.

Summary

The latest and most modern equipment is not always the answer to using plant labor efficiently. Using plant labor efficiently involves proper arrangement and balance of equipment, and use of proper work methods. Work methods can be developed by plant personnel through a "do-it-yourself" campaign. An analysis should be made, using the questioning attitude, considering the possibility of combining, eliminating, or simplifying operations, or changing the sequence of operations. Unproductive work should be eliminated; time saved should be devoted to productive work. Unproductive time by the worker is often the fault of management, for not providing proper work conditions and work place. So this is your challenge—set up the job as you would have it if you were carrying out the operation.

TECHNICAL NOTE

STUDY OF THE NATURALLY OCCURRING SALTS IN MILK

The salts that occur in milk in such amounts as to be of significance in relation to various physico-chemical properties of milk and other dairy products, consist of chlorides, phosphates, and citrates of potassium, sodium, calcium, and magnesium. These salts amount to approximately 0.90% of the weight of the milk.

It has been established that these salts exert a controlling influence on (a) the heat stability of milk *(29)*, (b) the stability of milk towards coagulation by alcohol *(27, 28)*, (c) age-thickening of sweetened condensed milk *(30)*, (d) feathering of cream in coffee *(27, 31)*, (e) coagulation of milk by rennin *(13)*, (f) clumping of fat globule on homogenization *(14)*, and possibly other phenomena.

The exact manner in which these salts function in the various phenomena has not been clearly revealed. Presumably they exist in milk largely in dissociated form, but the various ion concentrations have not been measured directly on the same samples. However, the known solubilities of calcium and magnesium phosphates and citrates suggest that milk may be saturated with respect to calcium, magnesium, citrate, and phosphate ions, and that these ions exist in equilibrium with an undissolved, dissolved, and undissociated excess of the corresponding salts.

Studies in the literature, in which concentrations of total and soluble calcium, magnesium, phosphorus, and citric acid in milk were considered simultaneously on the same sample of milk, were few and inadequate. The purpose of the present investigation was to study the nature of their simultaneous solution in milk, because such an examination was considered essential to a knowledge of the factors that influence the ionic equilibria in milk.

The various forms in which the salts occur in milk, such as ionic and colloidal, etc., have been known for a long time. In this study, in order to designate the various salts appearing in milk-sera obtained in various ways, the terminology used by the particular author cited has been retained in referring to his work. The term "soluble" has been used generally to designate the part appearing in milk-sera, but more specifically used to mean "present in whey." Van Slyke and Bosworth *(33)* studied the calcium phosphate of milk, using the Pasteur-Chamberlain filter to obtain casein-free serum. Other methods have been used in studying the soluble *vs.* insoluble and/or bound *vs.* free salts in milk. Dialysis in various forms as to membrane, dialysis liquid, temperature and time, has been used *(1, 18, 19, 22, 24, 36, 37)*. Kay *(17)* precipitated the proteins and fats by means of colloidal ferric hydroxide. Lenstrup *(20)* used picric acid precipitation and Sanders *(25)* used trichloracetic acid. Ling *(21)*, Anantakrishnan *(2)*, and Verma and Anantakrishnan *(35)* used rennet coagulation and the analysis of the whey to measure the soluble salts. Baker *et al. (3)* used an ion exchange resin-contact procedure to study the inorganic equilibria in milk.

These methods are not free from objections. Filtration methods such as used by Van Slyke and Bosworth *(33)* are extremely slow, and are open to criticism in that aging effects, acid development, and retention by absorption on the filter are complicating factors. Dialysis can hardly measure the equilibrium as it exists in milk. The use of precipitating reagents and filtrating alter salt solubilities by direct interaction or by a change in pH. The same objections would apply to resin exchange methods. Besides the adsorption of ions on the insoluble phase, centrifugal separation of the soluble and insoluble phases varies with respect to the time and force used. Rennet coagulation to obtain a clear filtrate may involve some slight change in the base-combining power of the casein, but this appears to be of minor importance in contrast to the more serious objections of the other methods. For this reason, also because of its simplicity and convenience—this method was selected for the present study, and was found to be quite satisfactory for comparative studies.

Experimental Procedure and Methods

Total Calcium: Thirty ml. 15% CCl_3COOH were added to 10 ml. of milk, mixed thoroughly, and filtered. Four ml. of filtrate were added to two ml. saturated $(NH4)_2C_2O_4$, neutralized to methyl red by adding dilute NH_4OH, then acidified to a faint pink color with dilute acetic acid. After heating in a boiling water bath for five minutes, the reaction mixture was allowed to stand at room temperature for eight hours. It was then centrifuged, the supernatant decanted off, and the precipitate washed three times, using five ml. dilute (1+9) NH_4OH each time, followed by centrifuging and decantation. The washed precipitate was dissolved in one ml. of dilute H_2SO_4 (1+1), and titrated at about 45° C. with 0.01 *N* $KMnO_4$ from a micro-burette until a faint excess of $KMnO_4$ persisted. Ignoring the volume of the fat and protein precipitate, the calculation is as follows:

$$\text{mg. Ca in one ml. milk} = \text{ml. of } 0.01\ N\ KMnO_4 \times 0.2$$

On the assumption that the volume of fat and protein precipitate amounted to 6.2 ml. per 100 ml. of milk, this value was corrected for the volume of this precipitate, by multi-

plying it by 0.985. The values compared well with determinations of calcium on the ash.

Total Magnesium: Four ml. of CCl_3COOH filtrate of milk from the calcium determination was taken in a 15-ml. graduated centrifuge tube and the calcium was precipitated as described above. Before centrifuging, however, the contents were made to the eight-ml. mark. After centrifuging, two ml. of the clear supernatant was pipetted into another centrifuge tube (representing 0.25-ml. of milk), and 0.5-ml. of five per cent ammonium phosphate (containing five ml. of concentrated NH_4OH per liter) was added with constant stirring, followed by two drops of concentrated NH_4OH. The contents were allowed to stand overnight and, after centrifuging, the supernatant was decanted off and the crystals washed very carefully three times with five ml. dilute (1+2) NH_4OH, each time centrifuging and discarding the washings. A final washing was given with five ml. 75% C_2H_5OH containing one per cent NH_4OH. After decanting, the tubes were allowed to stand (to allow the ammonia to evaporate) for three hours. Phosphorus was determined colorimetrically on the residue, according to Fiske and Subbarow *(10)*, and the amount of magnesium computed from that. A correction for the volume of fat and protein, similar to the one in the case of Ca, was made. The method was adopted from the one used by Denis *(8)* for Mg in blood serum.

Total Phosphorus: Five ml. of milk was made up to 100 ml. with distilled water in a volumetric flask and one ml. of this was digested with 2.5 ml. of 5 *N* H_2SO_4, followed by 0.5-ml. of perchloric acid; phosphorus was determined on the digest, according to Fiske and Subbarow (*loc. cit.*).

Total Citric Acid: Citric acid was determined exactly, according to the method of Deysher and Holm *(9)*.

Preparation of Whey: Milk was coagulated with rennet (0.1-ml. of commercial rennet extract per 100 ml. of milk) at 80° F. in a thermostatically controlled water bath, and after allowing 30 minutes for curd formation it was cut with a stainless steel spatula; whey thus obtained was filtered through a Whatman No. 40 filter paper. Soluble calcium, magnesium, phosphorus, and citric acid were determined on the whey, using the methods already described.

The per cent contents as found in the whey were converted to the corresponding per cent contents in the milk, by multiplying the former by the factor which had been found satisfactory for this purpose by Ling *(21)*, viz.,

$$\frac{100 - (\text{fat} + \text{protein} - 0.4)}{100}$$

Distribution of Calcium, Magnesium, Phosphorus, and Citric Acid: The amounts of calcium, magnesium, phosphorus, and citric acid in milk and whey were studied simultaneously on 15 samples of commercial raw milk obtained from the plant of the Department of Dairy and Food Industries at the University of Wisconsin. Results are presented (see Table 1).

Discussion

The amounts of calcium, magnesium, phosphorus, and citric acid in milk were studied simultaneously. The results of 15 such analyses are reported (Table 1). The amount of Ca varied from 116.4 to 174.8 mg. per 100 ml. of milk, with an average of 132.1 mg. per cent (184.9 mg. as CaO). The values compare favorably with the range of 175.0 to 190.0 mg. per cent CaO reported by Cranfield *et al. (5)*. The average value reported by Crichton *(6)* is 166.0 mg. per cent (120.0 to 212.0 mg.). Sommer *(27)* found it to range from 125.0 to 291.0 mg. per cent as CaO.

The values for Mg ranged from 8.4 to 15.4 mg. per 100 ml. of milk, with an average of 10.8 mg. (18.1 mg. as MgO). Van Slyke and Bosworth *(34)* reported the Mg content between 13.6 and 23.6 mg. per cent, while Sommer *(27)*, reporting as MgO, gave a range of from 5.0 to 39.0 mg. per cent. Values of 10 to 15 mg. per cent as Mg were reported by Lampitt and Bushill *(18)*.

Phosphorus ranged from 70.0 to 122.0 mg. per 100 ml. of milk, with the average being 95.8 mg. (219.3 mg. as P_2O_5) per 100 ml. of milk. Jacobsen and Wallis *(15)* found the P content in milk to be from 87.0 to 108.0 mg. per cent, and Burr and Witt *(4)* reported the P_2O_5 content from 168.0 to 275.0 mg. per cent. Van Slyke and Bosworth *(34)* found the P to range from 87.0 to 131.0 mg. per cent, and Graham and Kay *(12)* reported 103.7 mg. per cent as P.

The citric acid content ranged from 134.9 to 171.2 mg. per 100 ml. of milk. Sherwood and Hammer *(26)* reported citric acid values from 70.0 to 330 mg. per cent. The average citric acid content in this study is 156.6 mg. per cent and compares well with values reported in the literature.

The average whey-soluble Ca was found to be 51.8 mg. per 100 ml. of milk, which is 39.2 per cent of the total Ca content of milk. Thus, the colloidal calcium retained in the curd was 60.8 per cent of the total. This compares well with the range of from 54.3 to 75.0 per cent, reported by Van Slyke and Bosworth *(34)*. Study of the distribution of Mg gave the average soluble Mg content as 7.9 mg. per 100 ml. of milk, which is 73.2 per cent of the total. This compares with the range of 64.4 to 91.2 per cent for Mg filtered through the Pasteur-Chamberlain filter as reported by Van Slyke and Bosworth *(34)*.

The average for whey-soluble P was 36.3 mg. per 100 ml. of milk, being 37.9 per cent of the total. In comparison with the total contents in milk, Van Slyke and Bosworth *(33)*

TABLE 1

Distribution of calcium, magnesium, phosphorus, and citric acid in milk

Sample No.	Calcium		Magnesium		Phosphorus		Citric Acid		Inorganic (Ca/P) ratio in the curd[a]
	Total	Soluble	Total	Soluble	Total	Soluble	Total	Soluble	
	(mg. per 100 ml.)								
1	153.1	72.1	8.4	7.2	99.5	46.0	137.2	112.3	1.10
2	135.9	40.3	8.7	8.1	113.1	32.7	151.6	127.2	0.79
3	122.4	44.0	10.9	5.0	108.9	30.1	163.8	150.0	0.75
4	146.6	70.4	15.4	12.2	118.8	33.6	161.2	133.7	0.55
5	125.1	55.7	11.4	8.4	90.0	34.3	155.1	144.2	0.95
6	174.8	76.2	14.1	9.9	98.5	36.5	134.9	116.8	1.32
7	133.5	50.7	10.4	7.6	100.0	25.5	157.1	135.9	0.77
8	129.0	53.3	10.2	8.4	122.0	43.2	155.9	142.5	0.63
9	116.4	41.3	10.8	6.0	70.0	30.4	153.4	150.8	2.06
10	120.8	41.6	10.2	6.5	75.0	28.0	153.5	149.1	1.58
11	126.1	30.8	9.6	8.0	91.5	40.2	152.7	131.5	1.59
12	124.7	45.6	9.9	8.7	90.6	41.7	169.6	153.8	1.28
13	132.4	59.8	10.3	7.3	93.6	42.6	171.2	157.8	1.14
14	116.4	44.6	10.4	8.4	80.8	38.7	160.0	154.5	1.56
15	123.8	50.0	11.3	7.3	84.8	41.1	171.2	163.4	1.60
Av.	132.1	51.8	10.8	7.9	95.8	36.3	156.6	141.6	1.178
Per cent of total		39.2		73.2		37.9		90.4	.....
C.V.[b]	0.12	0.25	0.17	0.21	0.16	0.17	0.07	0.11	.....

[a] $\text{Inorganic (Ca/P) ratio in the curd} = \dfrac{(\text{mg. Ca in curd} - 25\text{ mg.})/40 + (\text{mg. of Mg in curd})/24 - 3/2\text{ m.moles of citric acid}}{(\text{mg. P in curd} - 18\text{ mg.})/31}$

[b] Coefficient of Variance $= s/\bar{x}$.

found the Pasteur-Chamberlain filtrate to contain from 27.0 to 64.3 per cent phosphorus, and the results compare well with these. In the literature, citric acid is cited as existing in solution *(7, 33)*. However, we have observed that about ten per cent citric acid is in colloidal state and does not appear in the whey prepared by the use of rennet. This fact is further established by observations made on pure salt mixtures, in connection with another phase of this study, in which the citric acid was in no instance in an entirely soluble state.

The mineral constituents present in a polyionic system such as milk are governed by the requirements of ionic equilibria, and present knowledge is far from adequate to deduce salt combinations for all the salts in milk. Several attempts have been made to define the combinations of inorganic calcium and phosphate in the insoluble and colloidal phase. The studies of Van Slyke and Bosworth *(33)* indicated the presence of colloidal $CaHPO_4$, and Pyne *(23)*, and de Kadt and van Minnen *(16)* concluded the combination to be tricalcium phosphate. Recent work of Ford *et al. (11)* also favored the presence of tricalcium phosphate in the casein colloid.

In the present study, the molar amounts of Ca, Mg, citrate, and phosphate in the curd were calculated after allowing for 1.63 per cent P_2O_5 *(32, 33)* and 1.5 per cent CaO *(16)* combining with natural casein. Thus, 25 mg. of Ca and 18 mg. of P were allowed for these combinations per 100 ml. of milk containing 2.5 per cent casein. Further, on the assumption that all the citric acid is present as tri-Ca- and/or tri-Mg salt in the curd, equivalent amounts of Ca-Mg were subtracted from total moles of Ca and Mg. The ratios of the moles of this inorganic (Ca + Mg)/moles of inorganic P (Table 1) were computed and out of the 15 samples, five samples showed a P:Ca ratio significantly above 1:1. All the others showed equal or less Ca than P; the average ratio of P:Ca for the 15 samples was 1.178. Variations in the ratios are wide and, while this by no means may be positive assurance of the exclusive presence of $CaHPO_4$, it seems to be the main form. Presumably, several combinations occur simultaneously, with dicalcium phosphate predominating in the curd.

Summary

Analyses were undertaken to study the amounts of Ca, Mg, P, and citric acid in samples of commercial milk, and the averages of 15 such analyses were 132.1, 10.8, 95.9, and 156.6 mg. per 100 ml. of milk, respectively.

Distribution of the above constituents into soluble (in whey prepared by use of rennet) and insoluble fractions was studied. The average whey-soluble Ca, Mg, P, and citric acid in milk were found to be 39.2, 73.2, 37.9, and 90.4 per cent, respectively, of the total amounts of these constituents in milk.

Molar ratios of inorganic colloidal Ca-Mg to inorganic P in rennet curd indicate a predominance of $CaHPO_4$ rather than of tricalcium phosphate.

I. S. VERMA[1]
H. H. SOMMER[2]
Department of Dairy and Food Industries
University of Wisconsin

[1] Present address: Lincoln University, Jefferson City, Missouri.
[2] Deceased.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEWS

185. Laboratory Manual for Dairy Microbiology. E. M. FOSTER AND W. C. FRAZIER. Burgess Publishing Co., Minneapolis, Minn. 62 pp. Mimeoprint. $2.50. 1957.

The revised edition of this laboratory manual will be welcomed by the many persons who have used and appreciated the former edition by the same authors. While no new experiments have been added, several revisions have been made in order to clarify, extend, or bring the procedures up to date.

This laboratory manual is designed to include the work of a class meeting in 30 to 32 laboratory periods of two hours each, at intervals of two and five days between periods. The experiments are divided into three parts: the first part, to acquaint the student with the microorganisms associated with milk and dairy products; the second, to examine the methods used in the control of milk quality, and the third, to study the microbiology of specific dairy products. Each experiment includes an introductory paragraph explaining the purpose or importance of the experiment, the experimental procedure, a few thought-provoking questions, and references to further information on the topic. For quick reference, a more detailed discussion of the procedures used throughout the manual is given in an appendix.

L. D. Witter

186. Mikrobiologie der Butter. KARL J. DEMETER. Eugen Ulmer, Stuttgart, Germany. 315 pp. 1956.

This handbook of butter microbiology is complete and current in every respect. Dr. Demeter has kept his material within the bounds of the title, and the information on butter-making processes, although extensive, is always pertinent to the understanding of some aspect of microbiology.

The book is divided into three sections. In the first section, general microbiology is covered, including the types, sources, and numbers of microorganisms in butter, buttermilk, milk, and cream. A detailed discussion of the more important organisms is given. The effect of microorganisms on the quality and keeping quality of butter comprises the second section, and the reactions and actions of both normal butter flora and contaminating organisms are dealt with. The third section correlates the microbiology of butter to actual butter-making practices, stating the various sources and types of contamination, the methods of general control, and specific suggestions for special problems.

Each of the three sections is followed by a discussion of the laboratory or plant methods employed for determining, analyzing, or evaluating the butter, butter constituents, and process procedures. Numerous tables, and 45 photographs and graphs, supplement and summarize the text.

An exceptionally extensive bibliography of cited literature is available, making this volume an unusually good source of reference material for further reading on the subject. Great care has gone into the preparation of a usable, well cross-listed subject index. L. D. Witter

187. La Fromagerie devant les Techniques Nouvelles. ANTOINE M. GUERAULT. Societe D' Edition Des Producteurs Agricoles Industriels et Coloniaux. 42 Rue Du Louvre, Paris -1. 300 pp. 2·200 Francs. 1956.

The subject matter of cheese manufacture in the light of new techniques is presented in four parts, with the following 16 chapter headings:

Part 1. General Aspects. Definitions and Regulations; Yields — Standardization — Coefficient G; Bacteria and Yeasts; Formation of Curd; Role of Calcium in the Coagulation of Milk by Rennet; Coagulation — Syneresis.

Part 2. Fresh Cheese. Definition — Methods of Manufacture.

Part 3. Soft Cheese Manufacture. Methods of Manufacture; Treatment of Soft Cheese After Manufacture; Curing of Soft Cheese; Natural Drainage of Soft Cheese; Soft Cheese Made from Cut Curd.

Part 4. Pressed Cheese. Pressed, but not Cooked — Saint-Paulin type; Pressed, but not Cooked — the Holland type; Pressed and Cooked Cheese — Using Pasteurized Milk.

Appendix — Tables of Yields and Standardization.

Because this book discusses the latest technical information regarding cheese manufacture

in France, it would provide graduate students in dairy technology with a good source of information about French varieties of cheese.

Wassef Nawar
S. L. Tuckey

BUTTER

188. Varying manufacturing methods for summer and winter butter. G. H. Wilster, Ore. State College, Corvallis. Milk Prod. J., **48**, 1: 14. 1957.

The author discusses Danish research work on the improvements of butter quality. As a result of the controlled quality program in Denmark, Danish butter has reached a high standard of excellence.

Studies and results of methods of butter manufacture for summer and winter seasons are discussed. The importance of temperature treatment of pasteurized cream, and the working of butter in a partial vacuum are discussed in relationship to the production of a butter with good body and texture. J. J. Janzen

CHEESE

189. Packaging. G. N. Fisher (assignor to Kraft Foods Co., Chicago, Ill.). U. S. Patent 2,777,770. 3 claims. Jan. 15, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 3:515. 1957.

A package for process cheese consisting of a number of wedge-shaped portions. The wedge-shaped pliable envelopes are placed in the package and the melted cheese filled into them. The closed package maintains the portions in the desired shape. R. Whitaker

190. Method and apparatus for the continuous production of cheese-forming curd. W. J. Harper and D. A. Seiberling (assignors, by mesne assignments, to The Ohio State University Research Foundation, Columbus, Ohio). U. S. Patent 2,781,269. 9 claims. Feb. 12, 1957. Offic. Gaz. U. S. Pat. Office, **715**, 2:353. 1957.

Milk containing starter and rennet is introduced into the bottom of a vertical column, where it is first agitated to thoroughly mix it, then brought to a quiescent state, to permit coagulation while moving upward. The curd is then cut and floated in a trough, where it is heated and drained. R. Whitaker

191. Method of forming a stack of interleaved cheese slices and apparatus therefor. R. G. Rudnik, R. E. Miller, and H. Borgardt (assignors to Kraft Foods Co., Chicago, Ill.). U. S. Patent 2,781,272. 14 claims. Feb. 12, 1957. Offic. Gaz. U. S. Pat. Office, **715**, 2:354. 1957.

Equipment is described for the manufacture of sliced cheese, the slices being separated by a sheet of paper, etc. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

192. Whey concentrate. E. Traisman and H. L. Fleming (assignors to Kraft Foods Co., Chicago, Ill.). U. S. Patent 2,781,267. 5 claims. Feb. 12, 1957. Offic. Gaz. U. S. Pat. Office, **715**, 2:353. 1957.

Whey is concentrated in a vacuum pan at about 140°, to a solids concentration between 50 and 65%. The concentrated whey is cooled in less than 1.5 minute with brisk agitation, to a temperature below 60° F. R. Whitaker

193. Gelled condensed whey and process. O. E. Stamberg (assignor to Consolidated Products Co., Danville, Ill.). U. S. Patent 2,780,548. 9 claims. Feb. 5, 1957. Offic. Gaz. U. S. Pat. Office, **715**, 1:165. 1957.

Whey containing the proteins in undenatured form is caused to form a gel by concentrating to 30 to 60% total solids and holding at a temperature below 90° F., in contact with 8 to 32% calcium lactate based on the weight of the whey solids, to effect gelation of the condensed whey in a time period of a few hours to several days. R. Whitaker

194. Ion exchange purification of whey in the preparation of lactose. M. E. Hull (assignor to Armour and Co., Chicago, Ill.). U. S. Patent 2,778,750. 3 claims. Jan. 22, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 4:763. 1957.

Fresh pasteurized whey is passed through a cation exchange material to reduce the calcium content to less than 0.02%, and then pasteurized and concentrated under conditions which do not tend to denature the proteins. The crystalized lactose is then removed.

R. Whitaker

DAIRY BACTERIOLOGY

195. A simple apparatus for measurement of gas production and activity of lactic starter cultures. W. E. Sandine, P. R. Elliker, and A. W. Anderson, Ore. Agr. Expt. Sta., Corvallis. Milk Prod. J., **48**, 1: 12. 1957.

The principal cause of the floating-curd defect of cottage cheese was shown to be gas-producing lactic acid bacteria in the starter cultures. Lactic acid bacteria capable of producing large quantities of carbon dioxide were isolated from the finished cheese and the starter culture when floating curd and excessive foam formation during the cooking stage of manufacture were encountered.

A simple, rapid laboratory test has been developed for testing starter cultures for gas production and lactic acid–producing activity. Starter cultures can be classified into low, intermediate, and high gas producers by this method.

The best solution to the problem of floating curd and excessive foaming in cottage cheese

manufacture appears largely to be a selection of cultures that are free from high carbon dioxide–producing lactic acid bacteria.

J. J. Janzen

DAIRY CHEMISTRY

196. Occurrence in butterfat of n-heptadecanoic acid. R. P. HANSEN, F. B. SHORLAND, AND N. J. COOKE. Nature, **179**, 4550: 98. 1957.

n-Heptadecanoic (margaric) acid, an odd-numbered, long-chain fatty acid, was demonstrated to be present in butterfat.

R. Whitaker

DAIRY ENGINEERING

197. New German vitaminizing process. J. GRINDROD, New Milton, England. Milk Plant Monthly, **45**, 8: 41. 1956.

Siemens-Schuckertwerke Aktiengesellschaft of Erlangen, West Germany, has developed a process of vitaminizing milk, by use of ultraviolet rays which make the process economically feasible. The equipment consists of 12 slightly inclined stainless steel tubes. Into each is coaxially built a rod-shaped, low-pressure mercury vapor lamp two meters in length. The milk flows through the tubes at a rate of 2,000 liters per hour, at a slight pressure of 0.8-atm, surrounding the radiation sources on all sides and completely filling the tubes. This prevents the formation of ozone, which has a detrimental effect on milk. Practically all of the radiation emanating from the mercury vapor lamps is used, because only very short lengths protrude outside the tubes, as a visual means of indicating whether the power is on or off. A cross section of the apparatus is given.

C. J. Babcock

198. New high-temperature pasteurization processes—instrumentation and control. H. B. ROBINSON, Div. of Sanitary Eng., Pub. Health Serv., Washington, D.C. Milk Food Technol., **20**, 9. 1957.

A temperature of 190° F. is not uncommon in pasteurizing milk products with momentary holding. Most of the lethal effect occurs during the heat-up period; therefore, both rates and final temperature must be carefully controlled to assure properly processed products.

H. H. Weiser

199. Higher temperature—shorter time. D. J. HANKINSON, Univ. of Mass., Amherst. Milk Plant Monthly, **46**, 1: 13. 1957.

There are indications that higher temperatures will not produce efficient pasteurization. The time-temperature relationship for destruction of bacteria, enzyme destruction, creamline destruction, and production of heated flavor is logarithmic. The advantages of higher temperatures and shorter times include the elimination of holding time standard, and reliance entirely on come-up time for lethality, improvement in flavor, and improved bacterial destruction. Data are presented relative to the contribution of each portion of the pasteurization cycle on phosphatase destruction, the temperature required for complete destruction of different test organisms at 0.25- and 0.50-second heating time and, the temperature necessary for phosphatase destruction and reactivation with 0.25- and 0.50-second heating time.

C. J. Babcock

200. Device for filling measured quantity of milk or other liquid. L. M. LEVINE (assignor to Mejur Corp., New York, N. Y.). U. S. Patent 2,779,351. 4 claims. Jan. 29, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 5: 926. 1957.

A bottom-up can-filler consisting of two measuring tanks connected to a three-way valve, which is manually operated to alternately deliver a measured amount of milk from the two tanks.

R. Whitaker

201. Plate-type heat exchanger. A. H. WAKEMAN (assignor to The Creamery Package Manufacturing Co., Chicago, Ill.). U. S. Patent 2,777,674. 7 claims. Jan. 15, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 3: 490. 1957.

A plate-type heat exchanger is described having corrugations in the plate so arranged to give maximum heat-exchange efficiency.

R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

202. For your truck's sake, cooperate. W. J. METZGER, Bowman Dairy Co., Chicago. Milk Plant Monthly, **46**, 1: 37. 1957.

Lack of cooperation between routemen and maintenance men can cost thousands of dollars. For example, driving a truck until the ring gear and pinion are ruined can increase the repair bill from $15 to $95, a loss of $80, or the profit on 24,000 qt. of milk. A simple clutch-pedal adjustment may save a complete clutch overhaul costing $30 to $65, or the profit on the sale of 9,000 to 19,500 qt. of milk. A plan is given to establish closer cooperation between routemen and garage foremen.

C. J. Babcock

203. The future of paper. G. L. HUFFMAN, Pur-Pak Division of Ex-Cello Corp. Milk Plant Monthly, **45**, 10: 27. 1956.

Dual packaging of milk in both single- and multiple-service containers will ultimately cease in favor of carton packaging exclusively, and the ½-gal. carton ultimately will supplant the standard unit for home use. C. J. Babcock

204. The future of glass. R. B. WILHELM, Owens-Illinois Glass Co. Milk Plant Monthly, **45**, 10: 26. 1956.

Packaging of milk in glass offers flexibility in package size and design to meet market requirements, and low cost due to the re-use factor. C. J. Babcock

205. Production controls can be simple. F. J. Spackman and G. E. Faierson. Milk Plant Monthly, **46**, 1: 26. 1957.

A daily report calculated in "gallons only" is described for use in production control in dairy plant operations. C. J. Babcock

FEEDS AND FEEDING

206. Lactase-hydrolyzed lactose in feed. E. G. Stimpson (assignor to National Dairy Research Laboratories, Inc., Oakdale, N. Y.). U. S. Patent 2,781,266. 14 claims. Feb. 12, 1957. Offic. Gaz. U. S. Pat. Office, **715**, 2: 353. 1957.

A feed for animals and poultry comprising lactase-hydrolyzed lactose, a milk product, a cereal, and a fat. R. Whitaker

HERD MANAGEMENT

207. Dairy-stable sanitation equipment. H. I. Myer and F. C. Myer. U. S. Patent 2,779,309. 7 claims. Jan. 29, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 5: 916. 1957.

A sensing element suspended over cows in a stanchion is activated when the cow arches its back; thereby automatically causing a chute temporarily to be placed immediately to the rear of the animal, for directing delivery of urine and fecal material to a designated point. R. Whitaker

208. Dairy-stable sanitation equipment. H. I. Myer and F. C. Myer. U. S. Patent 2,779,310. 4 claims. Jan. 29, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 5: 916. 1957.

Similar to U. S. Patent 2,779,309 (Abst. No. 207), with a pail or bucket instead of a chute, being positioned at the rear of the animal. R. Whitaker

209. Pipeline milking system and valve therefor. A. F. Gallistel (assignor to Perfection Manufacturing Corp., Minneapolis, Minn.). U. S. Patent 2,779,308. 1 claim. Jan. 29, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 5: 916. 1957.

Details are given for an easily cleaned pipeline milking system which includes a milk cooler. R. Whitaker

210. Animal stall having swivel-type, adjustable V yoke. P. K. Girton. U. S. Patent 2,779,312. 18 claims. Jan. 29, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 5: 917. 1957.

Details covering a yoke for holding cattle in a stall. R. Whitaker

211. Milking apparatus. L. J. Schilling (assignor to Schilling Electric Co.). U. S. Patent 2,777,420. 9 claims. Jan. 15, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 3: 424. 1957.

Details are given for the construction of a milking machine, including inflations, claw, and pulsation-creating mechanism. R. Whitaker

ICE CREAM

212. Basic formulation of special dietary frozen desserts. K. M. Beck, Abbott Laboratories, North Chicago, Ill. Ice Cream Rev., **40**, 6: 26. 1957.

Four different formulas for dietetic frozen desserts and two formulas for diabetic patients are presented. In addition, some of the labeling requirements for special dietary frozen desserts are given. W. J. Caulfield

213. Packaging ideas, plastic containers. D. Merlin. Ice Cream Rev., **40**, 6: 48. 1957.

A recent survey reveals that approximately 65½ milion plastic containers were used in 1955 for packaging ice cream and sherbets. This figure represents approximately four per cent of all the containers used by the ice cream industry. W. J. Caulfield

214. Ice cream disher. L. Myers. U. S. Patent 2,780,183. 1 claim. Feb. 5, 1957. Offic. Gaz. U. S. Pat. Office, **715**, 1: 73. 1957.

A hand-operated dipper for forming spherical-shaped individual portions of ice cream. R. Whitaker

215. Device for freezing ice cream and ices. T. Lo Faro. U. S. Patent 2,779,167. 1 claim. Jan. 29, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 5: 880. 1957.

A small portable freezer for making family-size quantities of ice cream, sherbets, and ices. R. Whitaker

216. Stick holder. A. Friedman (assignor to Joe Lowe Corp., New York, N. Y.). U. S. Patent 2,778,670. 4 claims. Jan. 22, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 4: 746. 1957.

A stick holder is described for inserting sticks in frozen novelties frozen in molds in a brine tank. R. Whitaker

217. Machine for the automatic molding and automatic delivery of ice cream. R. Hirschson. U. S. Patent 2,778,321. 27 claims. Jan. 22, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 4: 656. 1957.

A device is described for molding ice cream and placing it between two wafers. R. Whitaker

218. Printing-press for frozen edible sticks and sticks holding molded candy. D. P. Russey. U. S. Patent 2,778,298. 6 claims. Jan. 22, 1957. Offic. Gaz. U. S. Pat. Office, **714**, 4: 650. 1957.

A device is described for printing on sticks suitable for frozen novelties. R. Whitaker

MILK AND CREAM

219. Sterile cream for whipping. Anon. Milk Dealer, **46**, 2: 42. 1956.

Sterile cream for whipping is processed and packed under custom label by Avoset Co. of San

Francisco, Calif. In manufacture, cream is standardized to 30% fat, stabilized with 0.1 of one per cent sodium alginate, flash-sterilized, homogenized, and piped into sterile holding-tanks. In packaging, glass containers and vacuum-sealing bottle caps are heat-sterilized. Ultraviolet light is used to assure that the sterilized cream, caps, and bottles are never exposed to nonsterile air. The finished bottled product will keep for months under refrigerated storage. Temperature changes of nonrefrigerated storage affect the consistency of the cream. C. J. Babcock

NUTRITIVE VALUE OF DAIRY PRODUCTS

220. Feeding studies on vitamin- and mineral-fortified whole milk. L. J. Teply, R. F. Prier, and H. T. Scott, Wisconsin Alumni Research Foundation, Madison. Food Research, **21**, 6: 671. 1956.

The question of whether the regular consumption of milk, fortified with the daily quota per quart of several vitamins, along with recommended levels of minerals such as iron, copper, iodine, etc., might be harmful when used with a normal diet, was investigated. Rats were used in the feeding trials and the observations carried through several successive generations, observations being made on such factors as growth, reproduction, tissue changes, and general appearance. The conclusions were that no deleterious effects of the vitamin- and mineral-supplemented milk could be observed. F. J. Doan

221. Butterfat and heart disease. G. P. Whitlock, Dir. Health Ed., National Dairy Council, Chicago, Ill. Milk Prod. J., **48**, 1: 41. 1957.

The National Dairy Council is continually assembling available facts on butterfat and heart disease. Besides evaluation of published information, personal contact is maintained with colleges and universities, government agencies, and a number of allied industries, in order to follow experiments that are in progress. The NDC also sponsors new research at various institutions.

In order to cope with misleading statements concerning the possible relationship of fat to "heart disease," the NDC publishes "Dairy Council Digests" and "Nutrition News," in which various phases of nutrition are discussed. Releases to editors and science writers are made routinely by NDC, in an effort to keep the public fully informed on this controversial subject. J. J. Janzen

SANITATION AND CLEANSING

222. The performance of a detergent-sanitizer for milk utensil sanitation in unsupervised field tests. M. L. Speck and H. L. Lucas, Dept. of Animal Ind., N. C. State College, Raleigh. Milk Food Technol., **20**, 3. 1957.

A detergent-sanitizer was tested on 155 Grade A milk farms using customary methods of milk utensil sanitation. The result of this study favor the use of a detergent-sanitizer procedure. It is recommended as a routine method for lowering the bacterial count and keeping milkstone deposits at a minimum. H. H. Weiser

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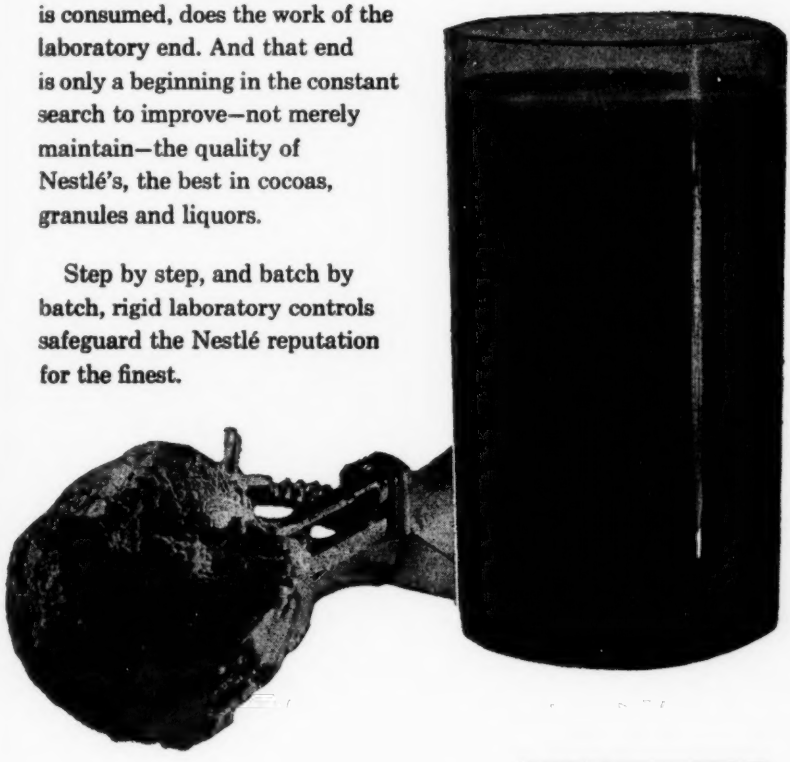

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